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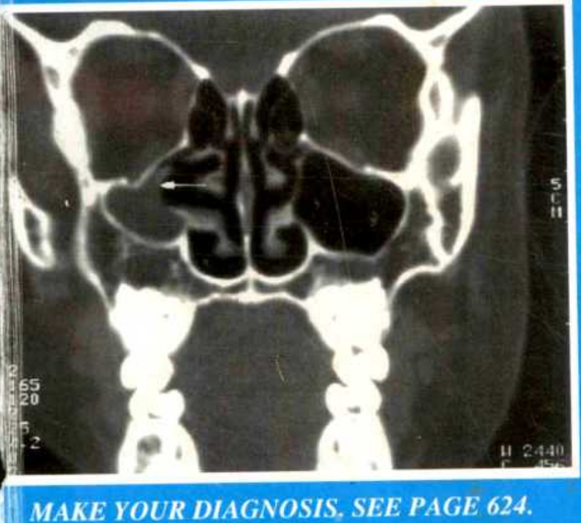

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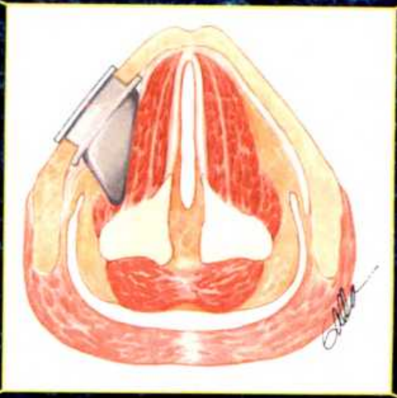

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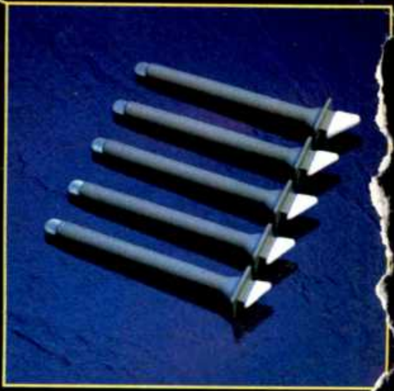

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THREE-DIMENSIONAL RECONSTRUCTION AND MEASUREMENT STUDY OF HUMAN EUSTACHIAN TUBE STRUCTURES: A HYPOTHESIS OF EUSTACHIAN TUBE FUNCTION

MASAHARU SUDO, MD

ISAMU SANDO, MD, DMSC CHIAKI SUZUKI, MD, DMSC

PITTSBURGH, PENNSYLVANIA

Histologic sections from nine temporal bones with eustachian tubes (ETs) attached were analyzed with a computer-aided three-dimensional reconstruction method to determine the curvature and tilt of the ET and the anatomic relationships between the medial lamina of the ET cartilage, the levator veli palatini muscle (LVPM), and Rosenmüller's fossa. It was found that the ET is convex superomedially and is tilted medially. The LVPM is located inferolateral to the inferior margin of the medial lamina of the anterior ET cartilage. The LVPM is inferior to the medial lamina of the posterior ET cartilage. These findings suggest that the LVPM opens the anterior cartilaginous portion of the ET by rotating the medial lamina with increased dimension of the muscle body when it contracts. Rosenmüller's fossa extends along the ET with rich lymphoid tissues as far as approximately one half the total length of the ET and very close to the skull base.

KEY WORDS — anatomy, auditory tube, computer-aided three-dimensional reconstruction, histology, human temporal bone, levator veli palatini muscle, pharyngeal recess, protective function, Rosenmüller's fossa, ventilatory function.

INTRODUCTION

The three-dimensional (3-D) anatomy of the eustachian tube (ET) is still not completely understood. Is the ET straight or curved along its longitudinal axis? Is the tube tilted or not, along its superior and inferior points? We performed a study to detail the shape and course of the ET to answer these questions. The other purpose of this study was to investigate the relationship of the ET lumen with the ET cartilage, the levator veli palatini muscle (LVPM), and Rosenmüller's fossa to test the hypothesis that the LVPM and Rosenmüller's fossa influence the ventilation of the middle ear (ME). The LVPM runs inferior to the ET cartilage and forms a muscle sling with fixed insertions at the petrous apex of the temporal bone and at the midline of the soft palate.[1,2] When the LVPM contracts, it is thought to elevate the ET cartilage, particularly its medial lamina, by the enlargement of the muscle belly, thus opening the pharyngeal orifice of the ET. Rosenmüller's fossa, located posteromedial to the ET, is thought to provide a space for the ET cartilage to be elevated by the LVPM, and thus for the ET to open. Further, Rosenmüller's fossa might have a more direct and closer relationship with ET function, because it lies close to the ET. However, little has been known to date about the precise shape, dimension, and location of the fossa and its interrelationship with the ET.

In this study, using our computer-aided 3-D reconstruction and measurement method, we reconstructed the ET, ET cartilage, LVPM, Rosenmüller's fossa, and apex of the petrous portion of the temporal bone from histology specimens and investigated their spatial interrelationships.

MATERIALS AND METHODS

Nine human temporal bone specimens with the ET attached were used for this study. Specimens had been obtained from nine individuals (16 to 88 years of age at death) with no history of ear disease or congenital anomaly. The specimens were fixed in 10% formalin, decalcified in 5% trichloroacetic acid, dehydrated in graded concentrations of ethanol, and embedded in celloidin.[3] The celloidin blocks with reference marks for computer reconstruction were sectioned at 30 µm as close to perpendicular to the axis of the ET as possible. Every 40th section was stained with hematoxylin and eosin and prepared for light microscopic study. Additional slides were prepared to compensate for the structural complexity of the ET and its surrounding structures in the junctional portion (isthmus; where the ET is surrounded by both bone and cartilage) and bony portion of the ET. These histology slides were then used for com-

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From the Elizabeth McCullough Knowles Otopathology Laboratory, Division of Otopathology, Department of Otolaryngology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania. This study was supported by Research Grant 5 R01 DC 00123 from the National Institutes of Health/National Institute on Deafness and Other Communication Disorders.

CORRESPONDENCE — Isamu Sando, MD, DMSc, Division of Otopathology, Dept of Otolaryngology, University of Pittsburgh School of Medicine, Suite 153, Eye & Ear Institute Building, 203 Lothrop St, Pittsburgh, PA 15213.

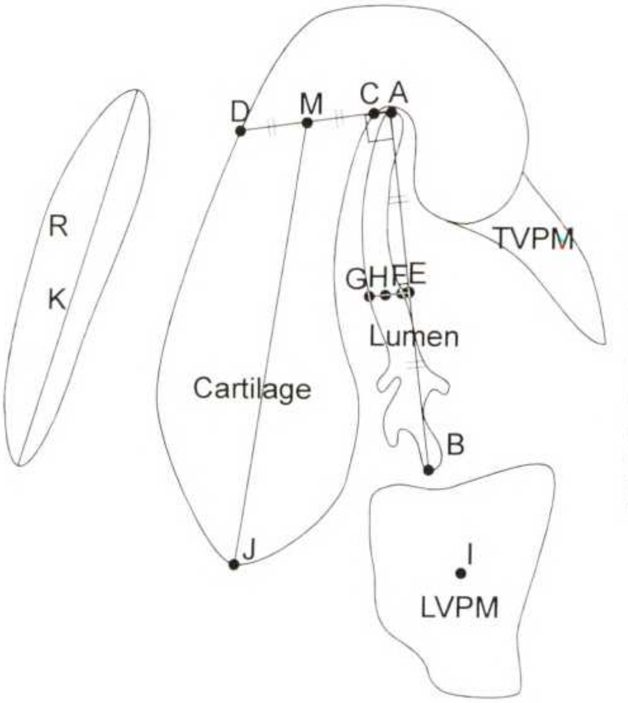

Fig 1. Schematic drawing of eustachian tube (ET) in cross section and surrounding structures in anterior view of left ET. A — most superior point of ET; AB — long axis of cross section of ET; AD — line perpendicular to line AB that passes through A; B — most inferior point of ET; C,D — intersections of line AD with margins of ET cartilage; EG — line perpendicular to and bisecting line AB; F,G — intersections of line EG with margins of ET; H — midpoint between F and G; I — center of gravity of levator veli palatini muscle (LVPM); J — inferior margin of ET cartilage, defined as most distant point from M; K — greatest dimension of Rosenmüller's fossa (R); M — midpoint between C and D; TVPM — tensor veli palatini muscle.

puter-aided 3-D reconstruction. The ET lumen, ET cartilage, and LVPM were reconstructed in nine cases. The epithelial line of Rosenmüller's fossa and the lymphoid tissue surrounding the fossa were reconstructed in seven cases.

The equipment we used for reconstruction consisted of a personal computer (NEC PC-9801RL), a digitizer (Hitachi HDG-1515, resolution 0.1 mm), and a printer. The software used for this study was developed by the first author (M.S.) to permit "virtual cuts" cross sections of the ET perpendicular to its longitudinal axis.[4]

On every virtual cross section, the long axis of the ET was first defined as the greatest dimension (the line AB in Fig 1). Then we defined the midpoint (H) of a perpendicular line that bisected the long axis of the ET (line EG in Fig 1). The longitudinal axis of the ET was defined as the straight line connecting the centers (H) of the cross sections between the pharyngeal and tympanic orifices; the pha-

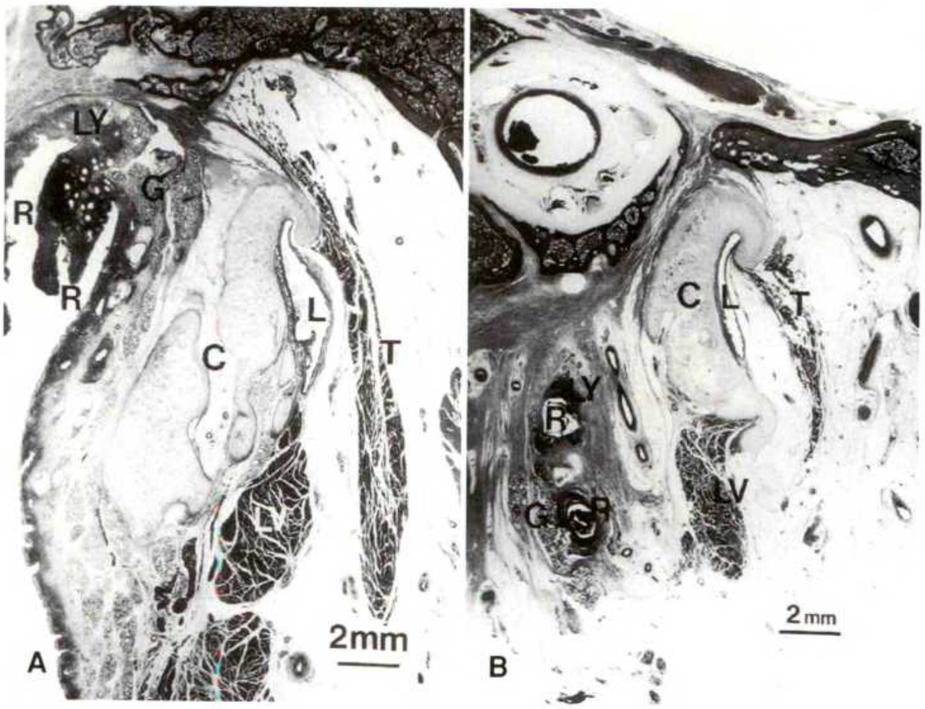

Fig 2. Photomicrographs of ET and surrounding structures stained with hematoxylin and eosin, showing areas A) near pharyngeal orifice and B) in MJ portion (midportion between midcartilaginous portion and junctional portion) of left ET in anterior view from 17-year-old girl with no history of ear disease. Right, left, superior, and inferior directions in Figures are oriented anatomically as anterolateral, posteromedial, superior, and inferior directions, respectively. Note relation between ET cartilage (C), ET lumen (L), levator veli palatini muscle (LV), and Rosenmüller's fossa (R). G — gland, LY — lymphoid tissue, T — tensor veli palatini muscle.

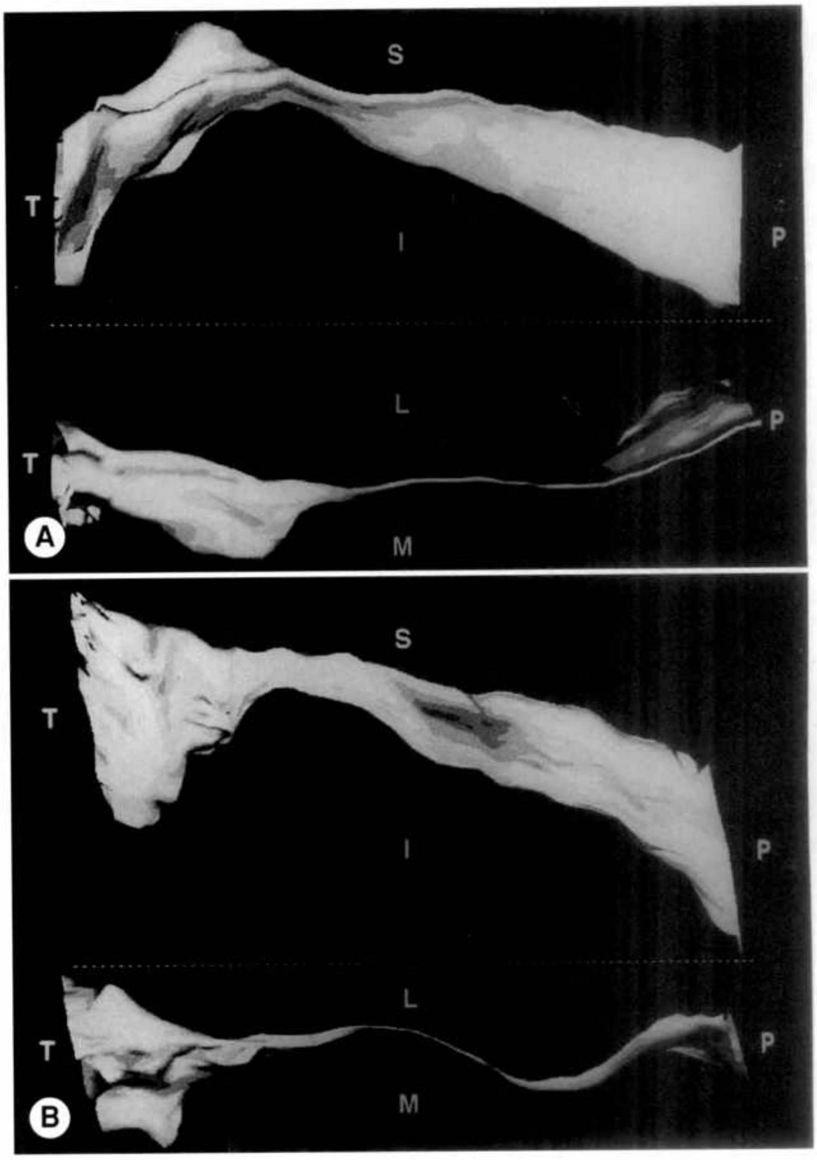

Fig 3. Surface model images of left ETs A) from 70-year-old man and B) 61-year-old woman. Upper and lower images represent posteromedial and superior view of ETs, respectively. Note that inferior margins of ETs curve upward in both cases; on other hand, superior margin in A curves upward, but other, in B, is almost straight (upper images). Lower images show how ETs curve horizontally. ET in A is gently convex toward medial, but other, in B, is rather sigmoid. I — inferior, L — lateral, M — medial, P — pharyngeal orifice, S — superior, T — tympanic orifice.

ryngeal orifice is located at the pharyngeal end of the tube, and the tympanic orifice at the anterior margin of the tympanic membrane.[4] The vertical (superior-inferior) direction in three dimensions was defined as parallel to line AB at the pharyngeal orifice. The location of the LVPM was represented by its geometric center in its cross section (I). Line AD in Fig 1 connected the most superior end of the ET (A), across the ET cartilage, with the outer margin of the ET cartilage (D), and was perpendicular to the long axis of the ET. Point C was then marked as the place where line AD crossed the inner margin of the cartilage. Point M was placed to bisect line CD. From point M, the most distant point of the medial lamina of the ET cartilage was defined as the inferior point of the ET cartilage (J). These points were marked on each virtual cut section of the ET, from the pharyngeal orifice to the tympanic orifice, along the length of the ET longitudinal axis automatically with our software to produce a file that was exportable to spreadsheet software (Microsoft Excel version 5 and 7, Microsoft Corporation) and graphing software (Sigmaplot version 3, Jandel Corporation) for data processing (normalization, averaging, etc).

We then analyzed the data for all sections to show the relationship between the ET, the inferior point of the medial lamina of the ET cartilage, and the LVPM along the longitudinal axis of the ET. Data for each structure mentioned were normalized to fit the averaged length of the structure along the ET longitudinal axis. When the long axis of the ET was tilted so that the superior margin of the ET lumen was located medial or lateral to the inferior margin of the ET, the computer program recorded this tilt in degrees as a function of the relative distance of that section from the pharyngeal orifice of the ET. The standard line for "0" value was the long axis of the

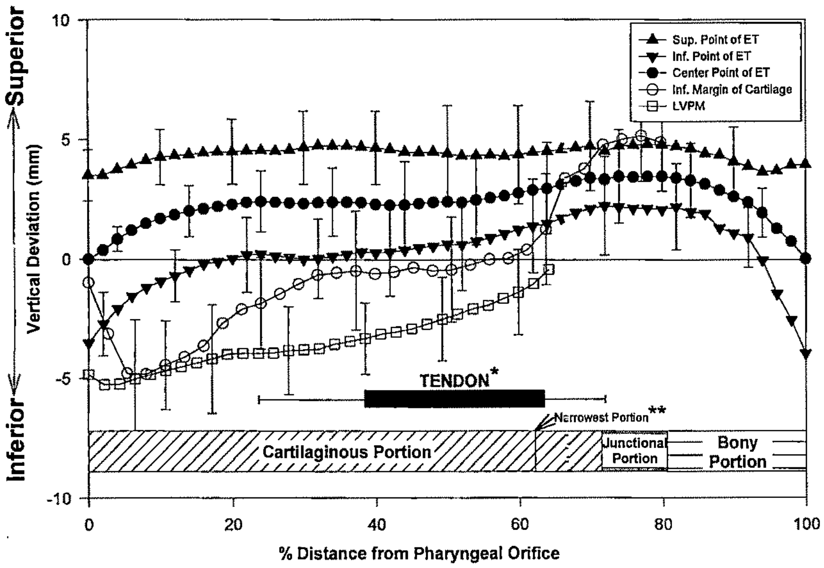

Fig 4. Vertical deviation of superior (triangles), center (circles), and inferior (upside-down triangles) points of right ET, inferior margin of medial lamina of ET cartilage (open circles), and center of gravity of levator veli palatini muscle (open squares), viewed from medial to lateral along longitudinal axis of ET. X-axis represents relative distance from pharyngeal orifice to tympanic orifice of ET. Y-axis represents vertical deviation in millimeters. Zero indicates longitudinal axis of ET from center point of nasopharyngeal orifice of ET to that of tympanic orifice of ET. Plus value in y-axis indicates upward deviation from axis. Error bars show standard deviation. * — tendon of tensor veli palatini muscle attached to lateral lamina of ET cartilage; ** — narrowest portion of ET when tubal muscles are not contracted.

ET at the pharyngeal orifice. For the left ear viewed from anteriorly, clockwise rotation (tilting) of the axis was assigned a minus value, and counterclockwise rotation a plus value.

On every virtual cut of Rosenmüller's fossa, the cross-sectional area for the surface surrounded by the epithelial line of the fossa, the circumference of the epithelial line, and the greatest dimension (K) of the surface surrounded by the epithelial line of the fossa were measured. Also, the cross-sectional area of the lymphoid tissues surrounding Rosenmüller's fossa was measured. The distance from Rosenmüller's fossa to the petrous apex of the temporal bone was also measured. Finally, we plotted these values as functions of the distance from the pharyngeal orifice of the ET.

RESULTS

Figure 2 shows photomicrographs of histologic sections of the left ET from a 17-year-old girl. The ET, ET cartilage, LVPM, and Rosenmüller's fossa are labeled. The LVPM appears inferolateral to the medial lamina of the cartilage near the pharyngeal orifice (Fig 2A). In the midportion of the ET between the midcartilaginous portion and the junction of the bony and cartilaginous portions[4] (MJ portion), the LVPM appears inferior to the medial lamina, which lies between the ET and the LVPM (Fig 2B). Rosenmüller's fossa is visible medial to the ET cartilage, surrounded by lymphoid tissue in the MJ portion as well (Fig 2B).

Although the ET curved upward in all nine cases, there was a variation in the curvature from case to case (Fig 3). For example, the ET from a 70-year-old man indicated that the superior margin of the ET curved (Fig 3A, upper image). On the other hand, the ET from a 61-year-old woman had a relatively

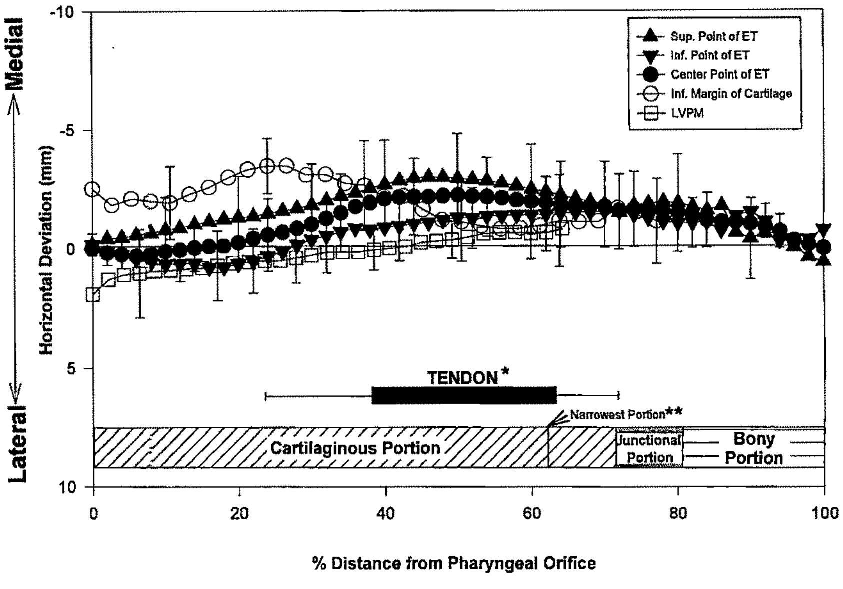

Fig 5. Horizontal deviation of superior (triangles), center (shaded circles), and inferior (upside-down triangles) points of right ET, inferior margin of medial lamina of ET cartilage (open circles), and center of gravity of levator veli palatini muscle (open squares), along longitudinal axis of ET. This Figure is compatible with superior view of left ET. X-axis represents relative distance from pharyngeal orifice to tympanic orifice of ET. Y-axis represents horizontal deviation in millimeters. Zero indicates longitudinal axis of ET from center point of nasopharyngeal orifice of ET to that of tympanic orifice of ET. Plus value in y-axis indicates lateral deviation from axis. Error bars show standard deviation. * — tendon of tensor veli palatini muscle attached to lateral lamina of ET cartilage; ** — narrowest portion of ET when tubal muscles are not contracted.

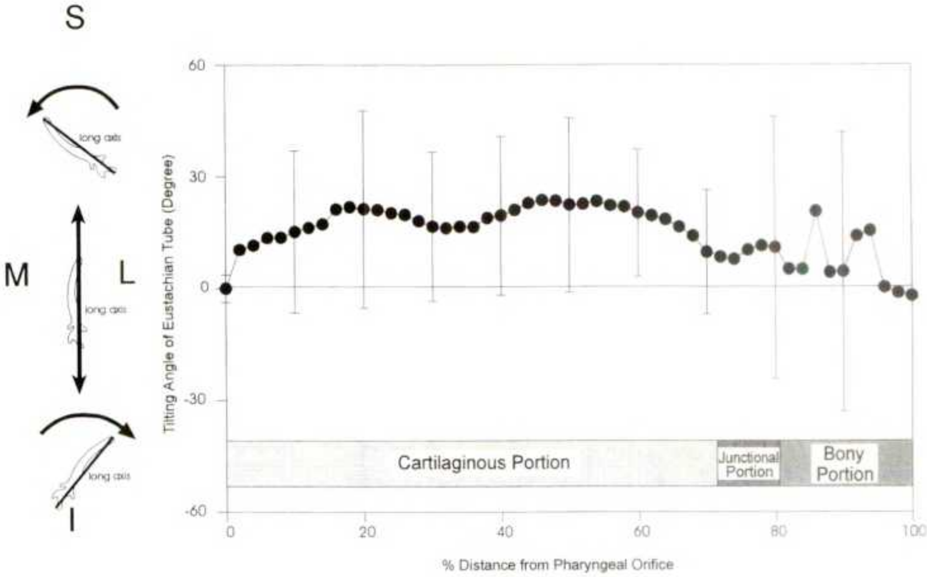

Fig 6. Angle of tilt of long axis of ET. X-axis represents relative distance from pharyngeal orifice to tympanic orifice of ET. Y-axis represents tilt in degrees. Zero indicates long axis of ET in its cross section at pharyngeal orifice of ET. Schema in left side of graph indicates how tilt of ET was defined. Counterclockwise rotation of long axis (line marked by "long axis") of left ET viewed from anterior represents plus value (upper Figure). Error bars show standard deviation. I — inferior, L — lateral, M — medial, S — superior.

straight superior margin (Fig 3B, upper image). Similarly, the ET curved gently medially in the former case (Fig 3A, lower image), but the curve was sigmoid rather than a simple bending (Fig 3B, lower image). In general, as Fig 4 shows, the ET curves upward — more so at its inferior margin than at its superior margin. The center point of the ET deviated most (3.5 mm) from the longitudinal axis, at 80% of the distance from its pharyngeal orifice (in the posterior end of the junctional portion of the ET). When viewed from superiorly, the ET appeared convex medially on average, rather than sigmoid (Fig 5). The maximal medial deviation of the center point from the axis was 2.24 mm, at 50% of the distance

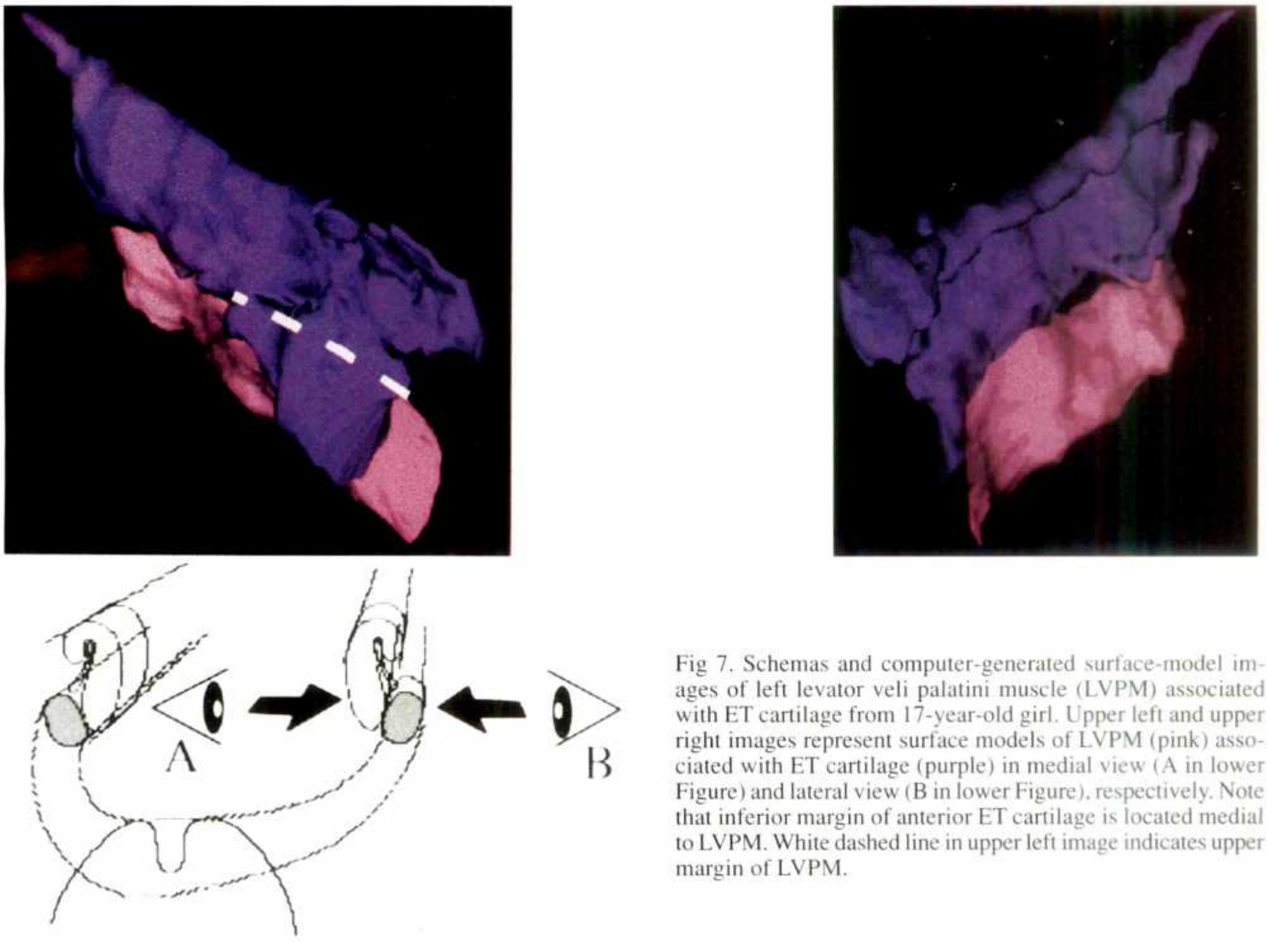

Fig 7. Schemas and computer-generated surface-model images of left levator veli palatini muscle (LVPM) associated with ET cartilage from 17-year-old girl. Upper left and upper right images represent surface models of LVPM (pink) associated with ET cartilage (purple) in medial view (A in lower Figure) and lateral view (B in lower Figure), respectively. Note that inferior margin of anterior ET cartilage is located medial to LVPM. White dashed line in upper left image indicates upper margin of LVPM.

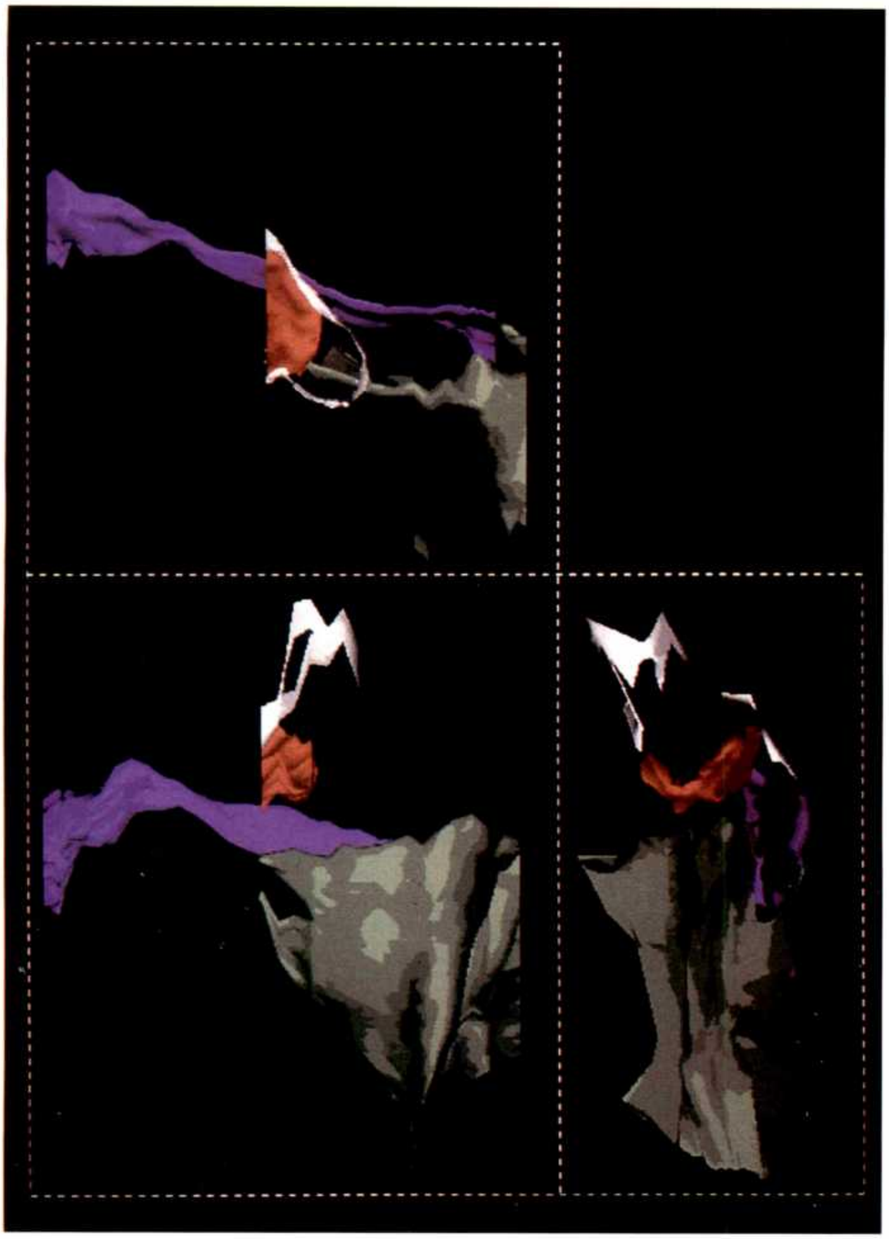

Fig 8. Surface model images of Rosenmüller's fossa (green), ET (purple), petrous apex of temporal bone (red), and foramen lacerum (white) in same case as in Fig 3A. Note that Rosenmüller's fossa is situated just posterior and parallel to ET and extends toward posterolateral direction as far as approximately half of total length of ET, and posterolateral end of fossa is located very close to petrous apex.

from the pharyngeal end, in the posterior half of the cartilaginous portion. Combining information from Figs 4 and 5, we may consider the ET to be convex superomedially. The long axis of the ET in cross section (line AB in Fig 1) was found to be tilted in relation to that at its pharyngeal orifice; the tilt was counterclockwise in the anterior view of the left ET. On an averaged graph (Fig 6), the maximal tilt of 23.6° was noted at 46% of the distance from the pharyngeal orifice, in the posterior half of the cartilaginous portion.

The computer-generated surface model images in Fig 7 represent the medial and lateral views of the ET cartilage and LVPM. In this case, the inferior margin of the cartilage was located posteromedial to the LVPM at the portion near the pharyngeal orifice, and inversely, it was located anterolateral to the LVPM at the posterior half of the ET cartilage. The LVPM was located inferolateral to the inferior point of the medial lamina of the cartilage throughout the anterior part of the cartilaginous portion of the ET (Figs 4, 5, and 7). More posteriorly, however, the LVPM was inferior to the inferior margin of the medial lamina of the ET cartilage, which separated the LVPM and the ET.

Rosenmüller's fossa was situated just posterior and parallel to the ET and extended toward the posterolateral direction as far as approximately one half (49.6% on average) the total length of the ET (Figs 8 and 9), and the posterolateral end of the fossa was located very close to the petrous apex (Fig 8). The shortest distance between Rosenmüller's fossa and

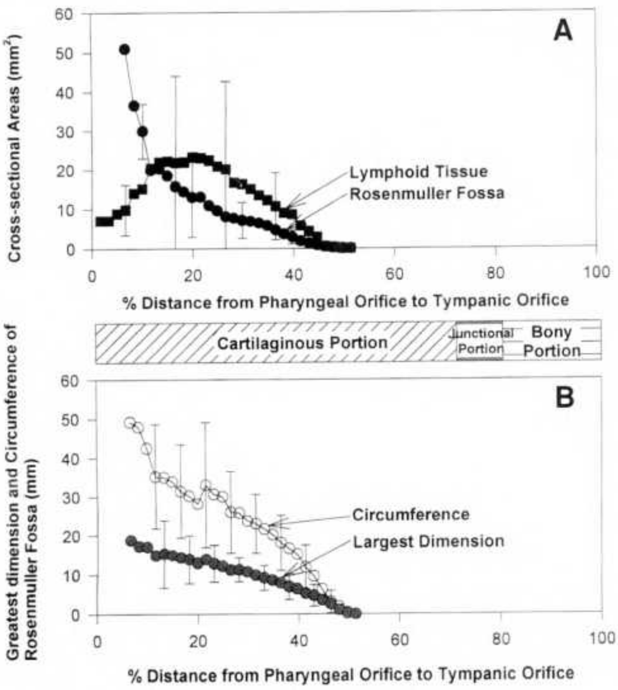

Fig 9. A) Cross-sectional areas of Rosenmüller's fossa (circles) and lymphoid tissue (squares) surrounding Rosenmüller's fossa. X-axis represents relative distance from pharyngeal orifice to tympanic orifice of ET. Y-axis represents cross-sectional area in square millimeters. B) Greatest dimension (closed circles) and circumference (open circles) of Rosenmüller's fossa. X-axis represents relative distance from pharyngeal orifice to tympanic orifice of ET. Y-axis represents dimension or circumference in millimeters. Note how deeply Rosenmüller's fossa extends into parapharyngeal space along ET. Error bars show standard deviation.

the petrous apex was only 4 mm on average in the seven specimens. The lymphoid tissue richly surrounded Rosenmüller's fossa (richest at 20% of the distance from the pharyngeal orifice), and it extended to the deepest part of Rosenmüller's fossa (Fig 9).

DISCUSSION

The LVPM originates at the inferior aspect of the petrous apex and inserts into the soft palate — not into the ET cartilage.[1,5] However, because the LVPM is located posterolateral to the inferior margin of the medial lamina (especially over the anterior half of the cartilaginous portion of the ET), the LVPM could influence the luminal shape of the ET[6]; it could open the pharyngeal orifice of the ET, by rotating the anteroinferior part of the medial lamina superomedially and posteriorly with the increased dimension of the muscle body when it is contracted (Figs 4 and 7). This might have an effect on the opening of the pharyngeal orifice of the ET.

On the other hand, opening of the posterior half of the cartilaginous portion of the ET (particularly the narrowest portion) may be closely associated with contraction of the tensor veli palatini muscle (TVPM), because the TVPM attaches to the lateral lamina of the ET cartilage in this region. Further, since the LVPM is located immediately inferior to the inferior margin of the medial lamina of the posterior portion of the cartilage, it cannot rotate the medial lamina superomedially; and the LVPM could not directly push the ET superiorly for opening, because the ET cartilage lies between the LVPM and the ET. The speculation that the LVPM and TVPM are closely associated with the opening of the anterior and posterior halves of the cartilaginous portion of the ET, respectively, may have implications for observations and experiments concerning the ET. The finding previously reported by Tomoda et al[7] that the TVPM has a greater population of white (fast) muscle fibers than the LVPM is consistent with the pumping function of the ET reported by Honjo et al.[8] First, the TVPM could open the posterior cartilaginous part and suck gas or fluid from the ME into the ET, and second, the LVPM could sequentially open the pharyngeal side of the ET. Difficulty in ventilating the ME in aircraft during descent is consistent with this ET opening function hypothesis (pumping theory), because the opening of the posterior half of the cartilaginous portion of the ET is expected to increase the pressure difference between the nasopharynx and the ME system during descent. More support is provided by the cineradiographic observation that electrical stimulation of the LVPM only opened the ET orifice, without producing effective ME ventilation.[9]

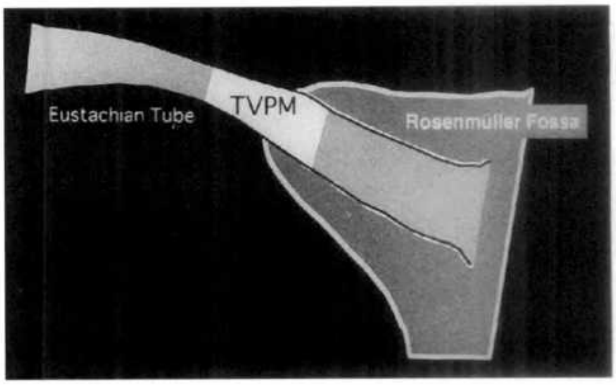

Fig 10. Illustration showing interrelationship between ET and Rosenmüller's fossa. Area for attachment of tensor veli palatini muscle to lateral lamina of ET is marked with white band.

Rosenmüller's fossa extended along the ET as far as approximately 50%, on average, of the total length of the ET (Figs 8 and 9). We previously found that the insertion site of the TVPM was at 38% to 63% of the length of the ET (Fig 10). So, this area is important for ET function, and it can be noticed that Rosenmüller's fossa and the lymphoid tissue reach this area, as viewed along the long axis of the ET. These results suggest that in case of upper respiratory tract infection, the lymphoid tissue might compress the ET at this area due to inflammatory swelling, causing ET dysfunction.[10] We also found that the shortest distance from Rosenmüller's fossa to the skull base is only 4 mm. Therefore, we can imagine that a nasopharyngeal carcinoma occurring in Rosenmüller's fossa can easily invade the skull base from this region.

The shape of the ET has not been thoroughly detailed in studies to date, and gross descriptions of ET shape have reported a degree of curvature that varies from almost straight[2] to acutely curved.[5,11] In the present study, we found that the ET is convex upward in adults (Figs 4 and 5). The varied curvature might be a result of differences in development of the ET as the mandible develops in childhood. The superior margin of the tube in our cases curved gently upward, and the inferior margin curved more distinctly upward; the superior margin was rather straight, and the inferior margin acutely curved. This difference between the inferior margin and the superior margin might be a reason for the variable reports. When viewed from superiorly (Fig 5), the ET appears convex medially, rather than S-shaped or straight, as claimed in former reports.[2,12] This curvature has implications for the ventilatory function of the ET: the medial convexity might help ventilate the ME, because it increases the angle of insertion of the TVPM into the lateral lamina of the cartilage, and could provide better traction on the lateral lamina. This hypothesis is based on the fact that the TVPM runs from the hamular process of the pterygoid bone, which is located in an inferoanterior direction from the ET, and inserts into the posterior half of the tubal cartilage at quite an acute angle of 20° to 30° (unpublished data).

Any study that involves histologic processing of specimens, including this study, may produce artifacts such as shrinkage of the section[13] (which has been estimated at 10% for the process used in this study) and distortion due to differences in shrinkage for different kinds of tissues.

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OTOLOGIC WEGENER'S GRANULOMATOSIS WITH FACIAL NERVE PALSY

PAUL DAGUM, MD, PHD
STANFORD, CALIFORNIA

JOSEPH B. ROBERSON, JR, MD
PALO ALTO, CALIFORNIA

Wegener's granulomatosis, characterized by necrotizing granulomas and vasculitis of the respiratory tract and kidney, frequently first presents with otologic symptoms. We report a case of primary otologic Wegener's granulomatosis in a patient who presented with symptoms of acute otomastoiditis and associated facial nerve palsy. The patient subsequently developed neuropathies of various cranial nerves. The patient underwent urgent mastoidectomy with facial nerve decompression. Nonspecific inflammatory disease of the mastoid mucosa delayed the correct diagnosis of Wegener's granulomatosis, which was confirmed by an elevated level of cytoplasmic-pattern antineutrophil cytoplasmic antibody (cANCA). We contrast the specificity of middle ear mucosal disease and cANCA serum levels in the diagnosis of Wegener's granulomatosis.

KEY WORDS — cranial neuropathy, facial palsy, Wegener's granulomatosis.

INTRODUCTION

Necrotizing granulomas and vasculitis of the respiratory tract and kidney characterize Wegener's granulomatosis. Friedrich Wegener first described this uncommon disease in 1936. The mean age of onset is approximately 40 years. Although it was once uniformly fatal, today, treatment with cyclophosphamide and prednisone achieves a 70% to 85% remission rate.[1,2]

Because Wegener's granulomatosis presents frequently with otologic symptoms, patients are often first evaluated by an otolaryngologist. A delay in diagnosis may lead to devastating sequelae, such as paralysis of the facial nerve, or extension of the inflammatory process to the skull base, resulting in multiple cranial neuropathies. Thorough familiarity with the subtle differences between Wegener's granulomatosis and other middle ear diseases should heighten the suspicion of this disease and expedite appropriate management.

CASE REPORT

A 59-year-old Filipino woman developed symptoms of an upper respiratory tract infection and right otalgia while visiting her daughter in Arizona. On returning home, she was initially treated for a presumed otitis externa. The following day, she developed right-sided facial weakness and was given prednisone. She underwent right tympanostomy tube placement and was subsequently hospitalized for approximately 1 week, during which time she received intravenous antibiotics. Middle ear cultures taken during tympanostomy tube placement were negative. The patient had persistent facial nerve palsy (House-Brackmann grade IV) and was discharged on a 10-day course of prednisone. Four days after discharge, the patient was readmitted for persistent right otalgia. A screening chest radiograph revealed multiple bilateral pulmonary nodules. A high-resolution computed tomography (CT) scan of the temporal bone revealed opacification of the right mastoid (Fig 1). The patient was treated with intravenous antibiotics without symptomatic or clinical improvement. On her third hospital day, she was transferred to our institution.

On arrival, the patient's physical examination findings were remarkable for a fever of 38.9°C, a very edematous and boggy right tympanic membrane with a whitish, thin, serous, mucopurulent effusion, a right facial nerve palsy (House-Brackmann grade IV), and bilateral basilar crackles. The rest of her examination findings were unremarkable. Audiometric evaluation was unsuccessfully attempted. The admission leukocyte count was 12,700/mm^3 with a 70% neutrophil fraction. A skull base, gadolinium-enhanced, magnetic resonance imaging (MRI) scan revealed right mastoid opacification and increased signal surrounding the right petrous apex. She underwent urgent right mastoidectomy. The mastoid mucosa appeared pale and edematous, and the mastoid air spaces were obliterated. Exposure of the facial nerve revealed poor perfusion of the nerve distal to the second genu. That segment of the nerve was decompressed. Blood immediately filled the nerve sheath

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From the Division of Otolaryngology–Head and Neck Surgery, Stanford University Medical Center, Stanford (Dagum), and California Ear Institute at Stanford, Palo Alto (Roberson), California.

CORRESPONDENCE — Paul Dagum, MD, PhD, Division of Otolaryngology–Head and Neck Surgery, Stanford University, Stanford, CA 94305-5328.

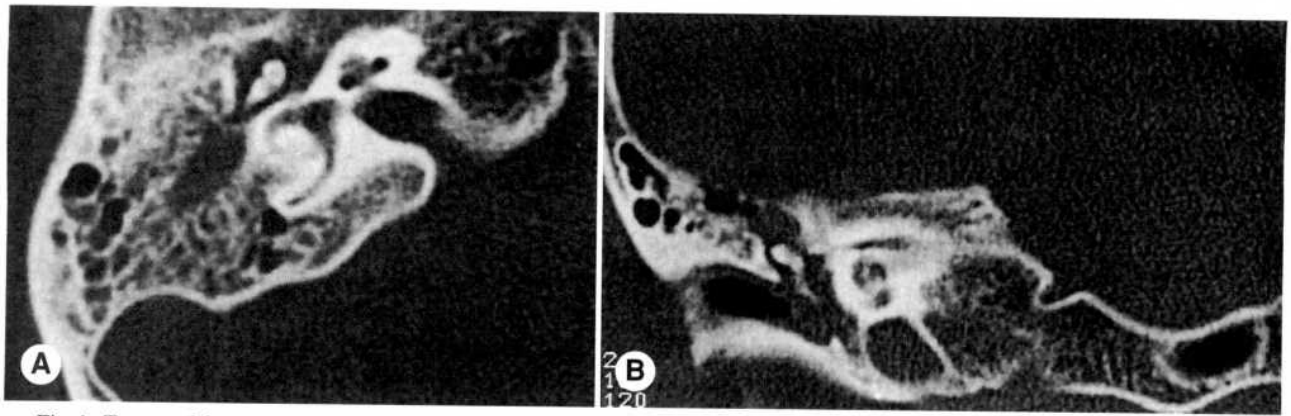

Fig 1. Temporal bone computed tomography. A) Axial view. B) Coronal view. Patient presented with this temporal bone computed tomogram, notable for opacification of mastoid and petrous apex air cells.

vessels, and the nerve enlarged to twice its original diameter. Extradural exploration to the petrous apex did not reveal subdural extension of the disease process. A tympanostomy tube was inserted on completion of the mastoidectomy and nerve decompression.

Mastoid mucosa was submitted for histology, acid fast bacteria (AFB) smear, Gram's stain, and aerobic, anaerobic, fungal, and mycobacterial cultures. Gram's stain showed scanty neutrophils, and the AFB smear and cultures were negative. Histology demonstrated only fibrinopurulent material admixed with chronic inflammatory cells.

Postoperatively, the patient's facial nerve function remained at a House-Brackmann grade IV. Her leukocyte count rose to 18,400/mm^3 and her fever reached 41°C. The patient was started on vancomycin hydrochloride and ceftazidime. Blood cultures were negative, a coccidioidomycosis titer was also negative, and a tuberculin skin test using purified protein derivative, and coccidioidomycosis and mumps controls, revealed anergy. An enzyme-linked immunosorbent assay of human immunodeficiency virus was nonreactive.

On the fourth hospital day, the patient developed hoarseness, decreased right-sided gag reflex, leftward deviation of the uvula, and rightward deviation of the tongue. Fiberoptic laryngoscopy confirmed a paretic right true vocal cord. A technetium leukocyte scan revealed intense activity extending from the right petrous apex to the jugular foramen. A repeat MRI scan revealed abnormal meningeal enhancement along the floor of the right middle cranial fossa. Cerebrospinal fluid cytology was normal, and fluid cultures were negative.

A chest CT scan revealed numerous cavitary lung masses measuring up to 4 cm and mediastinal lymphadenopathy. The patient underwent a CT-guided fine-needle aspiration of a pulmonary lesion. Cytology was notable for reactive epithelial cells, occasional multinucleated histiocytes, and marked polymorphonucleocytic infiltration. An AFB smear and stains with Giemsa's and Schiff's reagents were negative. The patient underwent bronchoscopy with lavage and transbronchial biopsy of a right middle lobe lesion. A milky fluid was obtained on bronchoalveolar lavage. Lavage culture grew 20 colonies of *Cryptococcus neoformans* and 10 colonies of *Candida albicans*. A cryptococcal antigen test, however, was negative, and the presence of oropharyngeal candidiasis was presumed to have contaminated the lavage. All other lavage tests, including aerobic and anaerobic cultures, mycobacterial cultures, AFB smears, and polymerase chain reaction for virus, were negative. Histology of the transbronchial biopsy showed mild, patchy, polymorphous inflammatory infiltrate of the alveoli consistent with a mild interstitial pneumonitis. Biopsy stains with hematoxylin and eosin and with Giemsa's reagent were negative.

On hospital day 8, the patient's facial nerve function degenerated to a House-Brackmann grade V. The patient underwent thorascopic lung biopsy, and again, histologic findings were unremarkable. A cytoplasmic-pattern antineutrophil cytoplasmic antibody (cANCA) test was positive in a dilution of 1:16. Antibiotic treatment was discontinued, and the patient received cyclophosphamide and prednisone. She had an almost immediate resolution of her fever and was discharged on hospital day 14, four days after commencing cyclophosphamide and prednisone treatment.

Examination of the patient 2 weeks after hospital discharge revealed resolution of cranial nerve IX and X palsies. She had brisk gag reflexes, a midline-rising uvula, and functioning vocal cords. She continued to have mild rightward deviation and weakness of her tongue, and her facial paralysis had not improved. Ear examination revealed a thickened, pale right tympanic membrane with a small inferior-

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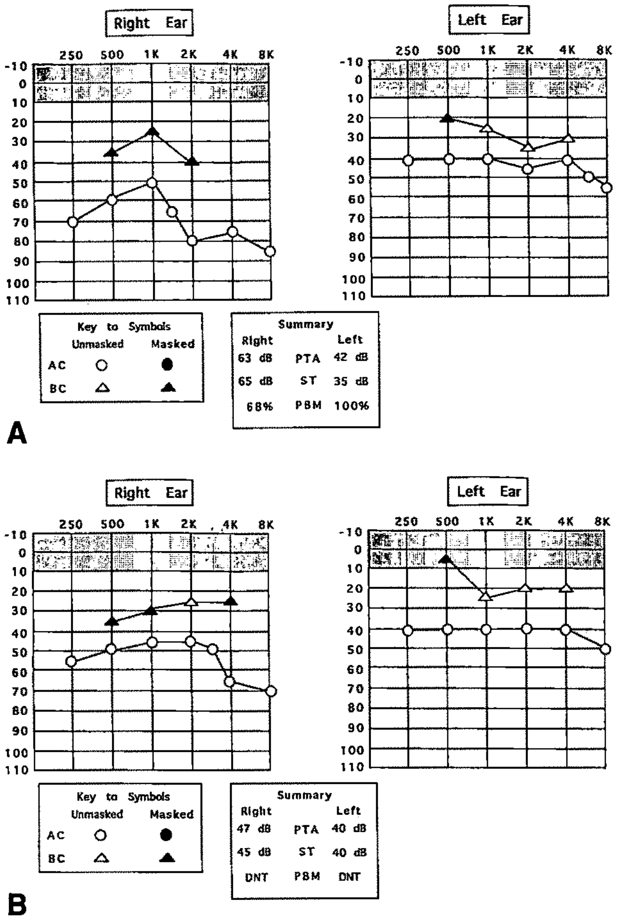

Fig 2. Audiograms at A) 2 weeks and B) 9 weeks after hospital discharge. We notice presence of both conductive loss (due to right tympanic membrane perforation, tympanic membrane thickening, and middle ear adhesions) and sensorineural loss (due to Wegener's granulomatosis nerve involvement). Air conduction (AC) thresholds improved with healing of perforation, and bone conduction (BC) thresholds improved after 9 weeks of cyclophosphamide and prednisone treatment. PTA — pure tone average of 500 Hz, 1 kHz, and 2 kHz AC thresholds; ST — speech threshold; PBM — fraction of correctly recognized phonetically balanced morphemes.

posterior retraction pocket and a small inferior-anterior perforation at the previous tympanostomy site. An audiogram showed bilateral mixed hearing loss (Fig 2A). Follow-up examination 9 weeks after hospital discharge revealed a healed right tympanic membrane perforation. A repeat audiogram showed a corresponding improvement in air conduction thresholds but persistent bilateral mixed hearing loss. Facial nerve function improved slowly and achieved a House-Brackmann grade II at 12 weeks after hospital discharge. At 1 year after discharge, her facial nerve continued to function at a House-Brackmann grade II level.

DISCUSSION

The ELK (ear, nose, and throat; lung; kidney) system[3] provides diagnostic criteria for Wegener's granulomatosis. Involvement of at least one of the three systems and either a biopsy confirming necrotizing granulomatous inflammation, usually with vasculitis, or the presence of cANCA confirms Wegener's granulomatosis.

Otologic manifestations occur in 19% to 45% of patients with Wegener's granulomatosis.[4,5] McCaffrey et al[4] classify otologic involvement into three major patterns: serous otitis media, sensorineural hearing loss, and chronic otitis. To this classification we add facial paralysis.

The majority of patients with otologic manifestations of Wegener's granulomatosis present with serous otitis media from eustachian tube dysfunction (ETD). Primary involvement of the nose and paranasal sinuses with nasopharyngeal inflammation is the presumed mechanism of ETD seen in patients manifesting serous otitis media. That group of patients invariably fail medical treatment for serous otitis media and may undergo tympanostomy tube placement. Persistent serous otitis media in an adult without prior ETD, however, should prompt a careful examination of the nasopharynx prior to insertion of tympanostomy tubes.

The mechanism of sensorineural hearing loss in Wegener's granulomatosis is unclear. Researchers, however, have postulated that vasculitic or granulomatous involvement of the auditory nerve is responsible for the hearing loss.[4,6,7] In contrast to serous otitis media, sensorineural hearing loss occurs from primary involvement of the ear by Wegener's granulomatosis.

Chronic otitis also occurs from direct involvement of the ear by Wegener's granulomatosis. Less than 10% of patients with otologic manifestations of Wegener's granulomatosis present with primary middle ear involvement. Findings may include necrotizing granulomas in the middle ear and mastoid, mixed hearing loss, and facial paralysis. McCaffrey et al[4] noted 1 case of facial paralysis in 5 patients with Wegener's granulomatosis of the middle ear, Kornblut et al[5] noted 4 cases with facial paralysis, and Macias et al[8] noted 1 case in 2 patients with this disease. In all cases, the facial paralysis resolved with treatment of the disease. In 2 patients with otologic involvement of Wegener's granulomatosis, Illum and Thorling[9] noted multiple neuropathies of cranial nerves VI, VII, IX, and XII. Both patients had large cranial base lesions with destruction of the petrous portion of the temporal bone. Cranial neuropathy arising from primary otologic involvement may be due to necrotizing vasculitis of the nerve or due to external compression, or neuritis, caused by granulomatous involvement of the middle ear.

Cranial neuropathy can also occur in the absence of primary otologic involvement of Wegener's granulomatosis. In a study of 324 patients with Wegener's granulomatosis, cranial neuropathy occurred in 21 patients.[7] Eight of these patients had facial nerve neuropathy. Neuropathy of cranial nerves IX, X, and XII occurred less frequently. A few patients had multiple cranial neuropathies. Researchers postulate that necrotizing vasculitis of the vasa nervorum produces cranial neuropathy in the absence of otologic involvement.

Early diagnosis of Wegener's granulomatosis demands a heightened suspicion of this disease in a patient with otologic symptoms and facial paralysis. Early treatment with cyclophosphamide nearly uniformly resolves the neuropathy.[5,10] A delay in treatment, however, may require mastoidectomy with facial nerve decompression to avoid permanent nerve damage. Because we can rarely make the proper diagnosis from biopsies of the middle ear mucosa,[2,10] clinical suspicion is paramount in the absence of a second organ involvement easily amenable to tissue diagnosis. As in the case presented, however, clinical symptoms may demand surgical exploration before a definitive diagnosis can be made. Histology can be misleading in primary otologic Wegener's granulomatosis, further delaying the diagnosis. In the case presented, retrospective reexamination findings of the mastoid mucosa and lung biopsy were considered consistent with Wegener's granulomatosis, but they were not typical for this disease, because they lacked well-formed epithelial granulomas or necrotizing vasculitis.

Clinical suspicion of Wegener's granulomatosis should prompt determination of cANCA levels[8]; cANCA corresponds to a specific cytoplasmic immunofluorescence staining pattern of antineutrophil cytoplasmic antibodies. In contrast to pANCA, a perinuclear immunofluorescence staining pattern, cANCA is 99% specific for Wegener's granulomatosis. The sensitivity of the test depends largely on the extent of the disease. For active, generalized Wegener's granulomatosis, the sensitivity approaches 93%.[11]

The most common initial diagnosis of otologic Wegener's granulomatosis appears to be otologic tuberculosis. Otolaryngologists have documented incidents of patients treated with antituberculous medications for otologic manifestations of Wegener's granulomatosis.[5,10] Test findings and multiple lung lesions common to patients with Wegener's granulomatosis are often also present in patients with tuberculous otitis media. Classically, a patient with tuberculous otitis presents with painless otorrhea, multiple tympanic membrane perforations that coalesce, and exuberant granulations.[12] Moreover, the histology of tuberculous otitis is diagnostic,[13] but Wegener's granulomatosis often shows nonspecific fibrinopurulence.

Otologic Wegener's granulomatosis may also mimic malignancy. Malignancy of the head and neck accounts for about 5% of all facial paralysis cases. Acute onset of paralysis occurs in approximately one quarter of tumor-related paralyses. More commonly, however, several weeks of progressive facial palsy evolves into a facial paralysis. Concomitant ETD from tumor involvement of the eustachian tube can lead to a serous or acute otitis media, obscuring the true cause of the facial palsy. Since malignant parotid tumors account for 80% of tumor-related facial paralyses, a proper examination of the head and neck with special attention to the parotid glands will uncover the majority of these cases. Other findings that suggest malignancy include otalgia and recent-onset sensorineural hearing loss. Further, the fact that malignancy is unlikely to cause both otomastoiditis and facial paralysis aids the otolaryngologist in distinguishing malignancy from Wegener's granulomatosis.

CONCLUSION

Wegener's granulomatosis is an insidious disease, and patients often first present to an otolaryngologist with otologic symptoms. Primary middle ear manifestation, without nasal or nasopharyngeal lesions amenable to diagnostic biopsy, delays the diagnosis. A thorough knowledge of the subtle differences between otologic Wegener's granulomatosis and more common causes of middle ear disease will aid the otolaryngologist in making the correct diagnosis. Facial nerve paralysis or other cranial neuropathy in the setting of chronic otitis media should heighten clinical suspicion and prompt a search for second organ involvement and determination of cANCA levels, after exclusion of more common causes.

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MANAGEMENT OF THE TINNITUS PATIENT

The Sixth Annual Conference on the Management of the Tinnitus Patient will be held in Iowa City, Iowa, September 18-19, 1998. Professionals who provide tinnitus management and patients who suffer from tinnitus are invited to attend. For information, please contact Richard Tyler, PhD, Director of Audiology, The University of Iowa Department of Otolaryngology–Head and Neck Surgery, 200 Hawkins Drive C21GH, Iowa City, IA 52242; telephone (319) 356-2471; fax (319) 353-6739; email rich-tyler@uiowa.edu.

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WEGENER'S GRANULOMATOSIS PRESENTING AS MASTOIDITIS

ABD EL-MATEEN MOUSSA, MD KAMAL A. ABOU-ELHMD, MD

SOHAG, EGYPT

Wegener's granulomatosis is an uncommon autoimmune disease. It has a peak incidence in the fifth decade of life, with a slight male predominance. Nasal problems are the predominant presentation, with rare otological manifestations. We report 2 cases of Wegener's granulomatosis presenting as mastoiditis in 2 young female patients. We conclude that young patients presenting with mastoiditis unresponsive to conventional medical and surgical therapy should be investigated for Wegener's granulomatosis.

KEY WORDS — mastoiditis, Wegener's granulomatosis.

INTRODUCTION

Wegener's granulomatosis was first described in 1936[1] as a necrotizing granulomatous disease affecting the upper and/or lower respiratory tract and associated in classic cases with focal glomerulonephritis. The nasal and paranasal cavities are usually the site of the presenting lesion.[2] Because of this, the otolaryngologist plays an important role in the early diagnosis and treatment.[3] Although its exact cause is unknown, it is assigned to the autoimmune diseases.[4] As it is a generalized disease, the kidneys, eyes, lungs, joints, skin, and nervous system may be involved. McDonald and DeRemee[5] reported head and neck involvement in 72.3% of their review of 411 patients with Wegener's granulomatosis. They also reported involvement of the lung in 71.2% and of the kidney in 57.5% in the same series. Otological involvement with Wegener's granulomatosis has been reported by many authors, but they usually do not describe it either as the initial problem or subsequent to nasal involvement.[6-12] However, 3 cases presenting with otitis media with effusion were reported by Karmody.[13] Serous otitis media was the initial manifestation of the disease in 7 cases in a review of 112 patients with Wegener's granulomatosis.[14]

This is a report of 2 cases wherein 1 presented with bilateral mastoiditis, and the second with unilateral mastoiditis.

CASE REPORTS

Case 1. A 14-year-old girl presented to our outpatient clinic with a history of right otalgia, fever, and sudden hearing loss. On examination, the right tympanic membrane was bulging with profuse pulsatile discharge. Movement of the pinna was not tender. The Rinne test was negative (512 tuning fork), but the Weber test was lateralized to the left ear. A diagnosis of masked mastoiditis was made, as she had had intensive antibiotics. Mastoid radiography showed some small clear cells, and pneumatization extended beyond the sinus plate on each side. An audiogram showed a right mixed hearing loss of 70 to 90 dB (Fig 1A) and a left conductive hearing loss of 30 to 60 dB (Fig 1B). No bacterial growth was obtained on culture of the discharge. The following day, myringotomy was performed and yielded purulent fluid bilaterally. She was discharged under medication with local and systemic antibiotics in the recommended doses. After 1 month, she returned with right otalgia. The following day, bilateral myringotomy with insertion of ventilation tubes was performed, and endoscopic examination of the postnasal space revealed no abnormality. Five months after the first presentation, she presented again with right otalgia and hearing loss. On examination, she was systemically ill and showed tongue ulceration, but otologically, no abnormality was detected other than the tubes and unchanged hearing loss. A diagnosis of Wegener's granulomatosis was considered. The general examination revealed no abnormality. The chest x-ray was normal, the serum creatinine was 71 µmol/L, and the serum urea was 6 to 7 mmol/L. The complete blood count was normal (the hemoglobin level was 12.3 g/dL, and the white blood cell count was 8.6×10^9/L). The differential count revealed no abnormality, with 74.2% granulocytes and 22.3% lymphocytes. The antineutrophil cytoplasmic antibody (ANCA) test was positive. She was admitted the following day for examination of the postnasal space and nasal cavities under general anesthesia, which revealed no abnormality. Two biopsy specimens were taken from the left inferior turbinate and the right anterior nasal septum. The histopathologic report showed no evidence of Wegener's granulomatosis. She was referred to a rheumatologist, who

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From the Department of Otolaryngology, Sohag Medical School, South Valley University, Sohag, Egypt.

CORRESPONDENCE — Kamal A. Abou-Elhmd, MD, Lecturer in ENT, Sohag Medical School, South Valley University, Sohag, Egypt.

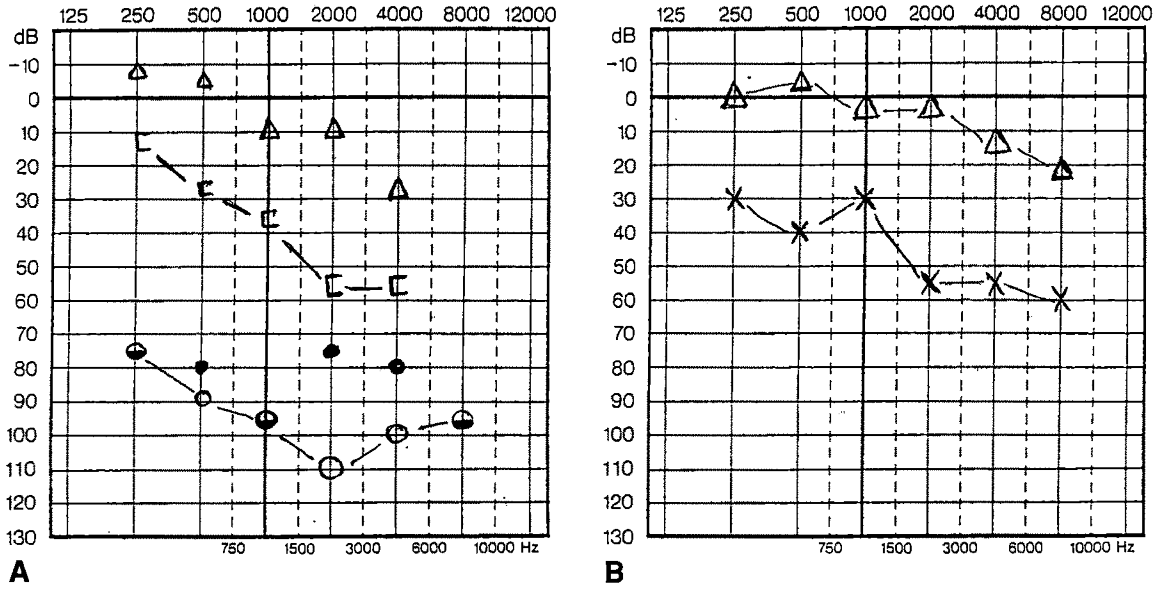

Fig 1. (Case 1) A) Right mixed hearing loss of 70 to 90 dB. B) Left conductive hearing loss of 30 to 60 dB.

commenced prednisolone 15 mg/d and aspirin 300 mg/d. In February 1992, renal biopsy showed crescents and patchy necrosis consistent with Wegener's granulomatosis. Cyclophosphamide and methylprednisolone were started. Unfortunately, after a sudden relapse 2 weeks later, her condition rapidly deteriorated and she died.

Case 2. A 20-year-old woman presented to our outpatient clinic with a history of fever, left earache and discharge, dizziness, tinnitus, nasal blockage, and occasional epistaxis. On examination, the nasal septum was severely deviated, and the left tympanic membrane showed the picture of serous otitis media. An audiogram showed a left conductive hearing loss of 70 dB (Fig 2) and a mild right hearing loss. She had been put on a waiting list for left grommet insertion and septoplasty. After 2 weeks, she returned with excessive purulent discharge from the left ear. Microsuction was performed, revealing a small central perforation, and chloramphenicol ear drops were prescribed. She was admitted to the hospital 1 month from presentation, and a grommet was inserted into her left ear. Unfortunately, following this, her ear continued to discharge. She was then seen again a month later, with a partial weakness of the left side of her face. There was a mucopurulent discharge through the grommet. The tube was removed, revealing polypoidal tissue extending through the tympanic membrane. Neurologically, she had left lower motor neuron partial facial palsy. She was admitted to the hospital the same day under medical treatment with erythromycin, oral prednisolone, and ear drops. She failed to respond to medical therapy and underwent a cortical mastoidectomy, revealing an antrum and aditus ad antrum full of granulation tissue and polyps, filling the mesotympanum. All granulation tissue and polyps were removed, and a specimen was sent for histology. The report showed no evidence of granuloma, no vasculitis, and no malignancy. A culture swab showed moderate growth of *Streptococcus faecalis*. The clinical chemistry report showed 3.2 mmol/L serum urea, 65 µmol/L serum creatinine, and 67 g/L total protein. Hematologically, the hemoglobin concentration was 13.4 g/dL, the granulocyte level was 77.5%, and the lymphocyte level was 18.2%. Ten days later, examination of the postnasal space and nose under anesthesia with a 90° Storz endoscope revealed no ulceration and no obvious lesion apart from a slight accumulation of mucus at the back end of the septum. Nonetheless, a biopsy specimen was taken under direct vision of the left eustachian tube and sent for urgent histology. This

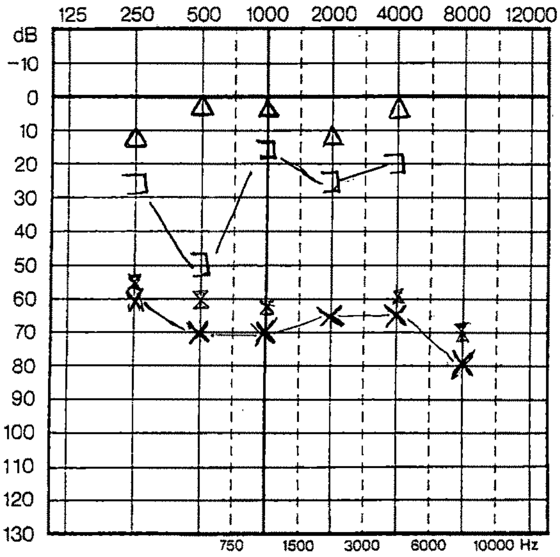

Fig 2. (Case 2) Left conductive hearing loss of 70 dB.

showed chronic inflammation with no evidence of granuloma or vasculitis. The right nasal cavity was reasonably clear, but the septum itself was very granular, so several specimens were taken for biopsy. On the left side, there was an adhesion between the septum and the inferior turbinate; a further biopsy specimen was taken for histology. Also, a biopsy specimen was sent for further culture, especially for acid-fast bacilli. The histopathologic report showed granulation tissues and inflamed connective tissue. Two weeks later, Wegener's granulomatosis was confirmed by a high titer of 1/150 positive ANCA. The patient was referred to a rheumatologist for further assessment and treatment.

DISCUSSION

Wegener's granulomatosis is an uncommon disease in adults, with a peak incidence in the fifth and sixth decades of life. It is rare in childhood, with a slight male predominance.[15] These 2 cases show that Wegener's granulomatosis may appear in young, female patients more than previously thought. It has a characteristic clinical presentation, with nasal and paranasal lesions predominately, but pulmonary and renal involvement can be considerable. Otological manifestations are almost always secondary to nasal involvement. The incidence of otological involvement varies widely, from 19% to 61%.[6] McDonald et al[16] divided otological involvement into three basic types.

1. Serous otitis media due to eustachian tube blockage and nasopharyngeal involvement.
2. Destruction of the middle ear and mastoid cavity by Wegener's granulomatosis.
3. A sensorineural defect due to inflammation of the cochlear vessels.

Its presentation as aural symptoms is uncommon, and for aural symptoms to be the only presenting problem is rare. Fauci et al[17] found only 5 patients out of 85 in their series complaining of aural symptoms. A case of Wegener's granulomatosis limited to the ear without involvement of the other systems has been reported.[18] In this case, the clinical picture and both laboratory and pathologic findings were consistent with Wegener's granulomatosis from the initial presentation.

To our knowledge, no one has described a presentation of Wegener's granulomatosis as mastoiditis. In our cases, in spite of early suspicions of Wegener's granulomatosis, we could not confirm it with the recommended investigations. It is recognized that Wegener's granulomatosis is a fatal condition, unless treated early, and its diagnosis depends on clinical and histopathologic features. Our cases are consistent not only with Wegener's opinion[19] that Wegener's granulomatosis can be diagnosed once the clinical features are compatible, but also with more recent research that concluded that a complete or typical histologic picture is not always essential to a diagnosis of Wegener's granulomatosis, if the clinical picture is suggestive of the diagnosis.[20] However, we could not depend on the clinical picture only, as many diseases may have the same features.

We suggest that if a patient presents with mastoiditis unresponsive to conventional medical and surgical therapy after more than 1 month, the diagnosis should be reconsidered. A diagnosis of Wegener's granulomatosis should be borne in mind if investigations exclude diabetes, tuberculosis involvement of the ear, sarcoidosis, and other autoimmune diseases. If the findings of recommended investigations, including high sedimentation rate, positive ANCA test, proteinuria, abnormal serum creatinine level, anemia, and nasal biopsy (as the nose is the earliest organ to be involved, usually, and it is a much easier and safer site for a biopsy) fail to confirm it, a middle ear mucosal biopsy specimen should be taken for histopathologic analysis, and if this also fails, kidney biopsy should not be delayed.

Diagnosis of Wegener's granulomatosis, when it is limited to the ear, should be established very early to avoid or lessen the degree of irreversible renal damage.

ACKNOWLEDGMENTS — We thank L. Flood, FRCS, M. Hawthorne, FRCS, and F. Martin, FRCS, ENT Department, North Riding Infirmary, Middlesbrough, England, for allowing us to include their patients in this report and for their support and guidance. We also thank Claudia Godfrey and Ear Nose Throat and Eye Research, in the same institute, for their help.

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XXTH REGULAR MEETING OF THE BARANY SOCIETY

The XXth International Barany Society Meeting will be held in Wurzburg, Germany, September 12-15, 1998. For more information, contact Prof. Dr. med. C.-T. Haid; telephone 0049-971-64832; fax 0049-971-68637.

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RECENT ADVANCES IN OTITIS MEDIA

The 7th International Symposium on Recent Advances in Otitis Media will be held June 1-5, 1999, in Fort Lauderdale, Florida. For information, contact David J. Lim, MD, House Ear Institute, 2100 W Third St, Los Angeles, CA 90057.

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EPITHELIAL DAMAGE OF NASAL MUCOSA IN NASAL ALLERGY

KENSUKE WATANABE, MD CHÔSEI KIUNA, MD

KOSHIGAYA, JAPAN

Epithelial alterations arising from moderate nasal allergy to house dust were examined and compared to findings in epithelia from nonallergic controls. Biopsy specimens were taken during natural allergen exposure from two different sites: 1) the anterior tip of the inferior turbinate and 2) 2 cm behind it. The tissues were examined by both electron and light microscopy. In the allergic group, epithelial damage was found to be remarkable in the anterior nasal mucosae, where nonciliated cells were prevalent, but minor in the posterior nasal mucosae comprising ciliated and goblet cells. In the anterior nasal mucosae, conspicuous intercellular edema, epithelial shedding, and clusters of eosinophils in the epithelial layer were observed, whereas only a little epithelial shedding and edema in the basal area of the epithelium was noted in the ciliated areas. In controls, pathologic changes were not observed, although a little epithelial shedding was seen in the anterior turbinate. Although there are arguments for and against epithelial shedding in nasal allergy, this study confirms its presence even in patients with moderate allergy.

KEY WORDS — electron microscopy, eosinophil, epithelial damage, nasal allergy.

INTRODUCTION

The damage to the bronchial epithelium of the patient with asthma is well known. The epithelial damage is a prominent postmortem finding in asthma,[1,2] but bronchial biopsy specimens have revealed that it may also occur even in subjects with mild asthma.[3,4] Further, epithelial damage contributes to bronchial reactivity.[4,5] The degree of epithelial loss is reported to correlate with the degree of airway reactivity.[4] In nasal allergy, some researchers have reported that epithelial shedding is also observed,[6,7] while others have indicated that the nasal epithelium remains almost completely intact with no concomitant epithelial shedding.[8,9] The obvious question thus arises as to why such a discrepancy exists regarding pathologic changes presumed to take place in nasal mucosa. In an effort to resolve this issue, the present study reexamines damage to the nasal epithelium in nasal allergy.

Different types of epithelial cells are known to be present throughout the inferior turbinate. Notably, for example, in the anterior tip, the epithelium is nonciliated, while farther back on the inferior turbinate, the epithelium becomes ciliated.[10-13] Under ordinary circumstances, the nose is the first target for airborne attack and hence the first defensive line against ambient air, characterized by its unphysiological temperature and humidity and inclusion of microorganisms and allergens. In particular, most of the inhaled particles are deposited on the anterior third of the nasal cavity, and it is changed by these adverse stimuli to such a degree that it differs from the rest of the mucous membrane.[10,14] Therefore, the anterior tip of the inferior turbinate may have a distinct role in activating the host's defense mechanism as compared with the more posterior area. In the present study, mucosal biopsy specimens were taken during natural allergen exposure from two different areas of the inferior turbinate — the anterior tip, and the posterior area — and the differences in epithelial damage between these anterior and posterior inferior turbinate areas were compared in allergic individuals naturally exposed to house dust and nonallergic control subjects.

MATERIALS AND METHODS

Biopsy specimens were taken of the mucosa of the inferior turbinate of six patients afflicted with nasal allergy who had a positive radioallergosorbent test (RAST; score ranged from 2 to 3) and a positive skin prick test to house dust, and of four control subjects who had no nasal symptoms and a negative RAST. The patients ranged in age from 20 to 46 years, and the controls from 15 to 45 years. The control subjects were normal, healthy, nonallergic volunteers from the clinic. Each participant gave informed consent. All patients were considered to have moder-

TABLE 1. DEFINITION OF MODERATE SYMPTOMS

	Sneezing			
Nasal Obstruction	+++	++	+	–
+++	S	S	S	S
++	S	Mo	Mo	Mo
+	S	Mo	Mi	Mi
–	S	Mo	Mi	N

S — severe, Mo — moderate, Mi — mild, N — no symptoms.

From the Department of Otorhinolaryngology, Koshigaya Hospital, Dokkyo University School of Medicine, Saitama, Japan.

CORRESPONDENCE — Kensuke Watanabe, MD, Dept of Otorhinolaryngology, Dokkyo University, 2-1-50 Minami-koshigaya, Koshigaya, Saitama 343, Japan.

TABLE 2. RATING SCALE FOR QUANTITATIVE ANALYSIS

	+++	++	+	–
Sneezing	>10 times per day	9-5 times per day	<4 times per day	None
Nasal obstruction	Inability to breathe through nose all day long	Inability to breathe through nose several times per day	Breathing through nose, but feeling of nasal obstruction	No nasal obstruction

ate nasal allergy, which was defined according to Tables 1 and 2. Biopsy specimens were taken during natural allergen exposure to house dust. At the time of examination, no patients had used any medication or experienced respiratory infection for at least 1 month prior to the study. All patients received submucous resection of the nasal septum and conchotomy under general anesthesia. Biopsies were taken from two sites: the anterior tip of the inferior turbinate (from anterior tip to 1 cm behind it) and 2 cm behind the anterior tip. To prevent the occurrence of artificial damage to the epithelium, the mucous membrane was sharply cut off by surgical knife and taken out without directly touching the epithelial surface. Small blocks were fixed for 2 hours by immersion in ice-cold 2% glutaraldehyde buffered with 0.1 mol/L cacodylate, pH 7.3, and then washed three times with 0.1 mol/L cacodylate buffer (containing 8% sucrose, pH 7.3). They were then post-fixed for 40 minutes in 2% osmium tetroxide and embedded in Epon 812. Ultrathin cross sections were prepared with a microtome (RMC MT 6000 XL), stained with uranyl acetate and lead, and examined by transmission electron microscopy. For light microscopic observation, 1-μm-thick sections of the tissues embedded in Epon were cut and stained with alcoholic toluidine blue.

RESULTS

In the anterior tip of the inferior turbinate (allergic patients), the epithelial surface featured microvilli but not cilia, and was composed of five to six layers of flattened epithelial cells. Different grades of epithelial damage were observed. The slightest damage noted was the shedding of the outermost one or two layers of stratified epithelium (Fig 1). The outermost epithelial cells had degenerated and were swollen (Fig 2). Beneath the first epithelial cell line, edematous fluid was observed (Fig 2). Eosinophils (Fig 2) were observed in the intercellular spaces. Some eosinophils showed frank disruption, with free granules in the space between epithelial cells (Fig 2, arrowheads). In the advanced stage of damage to the epithelium, intercellular edema was distinctly observed by light microscopy (Fig 3). The surface of the epithelium protruded into the airway space, and two or three of the outermost epithelial layers had degenerated (Fig 3, arrowhead). Finally, the epithelial cells became detached and were destroyed (Fig 4). On electron microscopy (Fig 5), destruction of the epithelial cell junction (arrowhead) and a cluster of eosinophils (double arrowheads) were noted.

Two centimeters behind the anterior tip of the inferior turbinate (allergic patients), the epithelial layer was composed of ciliated cells and goblet cells, but stratified columnar epithelium with microvilli was observed in the transitional zone from the nonciliated area to the ciliated area. Even though a wide intercellular space was observed in this transitional zone (Fig 6), the epithelial cells appeared to adhere together at the tight junctions (Fig 6, arrowheads). Epithelial damage was slight in the ciliated area (Figs 7 and 8), where almost the same numbers of eosinophils were observed as in the nonciliated area. No shedding of epithelium was observed. If a wider area was searched, however, edema fluid was seen to be present between the basal cells and the upper epithelial layers (Fig 7, arrowhead). At the surface of the epithelium, many goblet cells were found and no intercellular edema was noted (Fig 8).

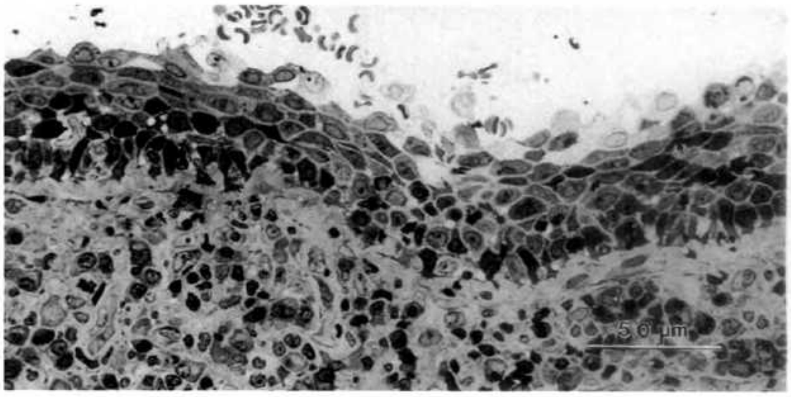

Fig 1. Epithelium of anterior tip of inferior turbinate is composed of five or six layers of flattened epithelial cells. Epithelium is slightly damaged. Outermost one or two epithelial cells have been shed.

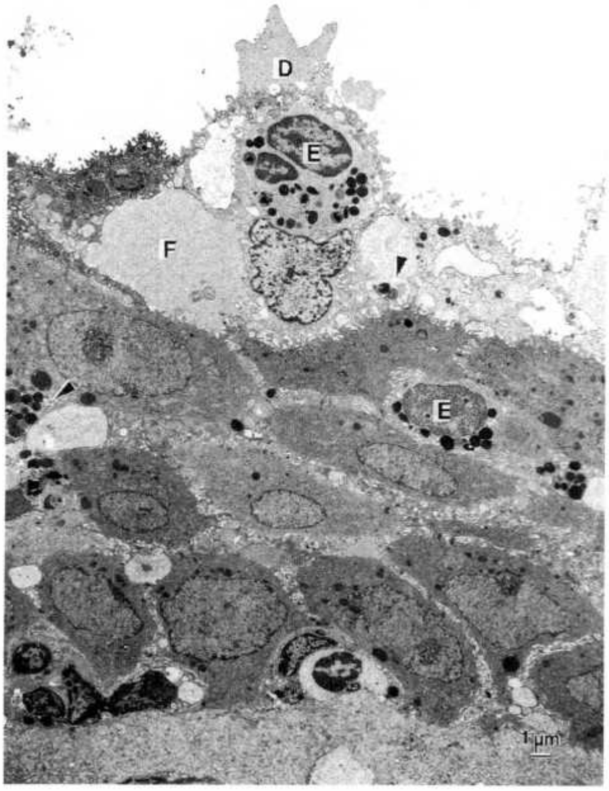

Fig 2. This electron microscopy photograph shows slightly damaged epithelium of anterior tip of inferior turbinate. Outermost epithelial cell is degenerated and swollen (D). Many eosinophils (E) and edema fluid (F) are observed in wide intercellular spaces. Some eosinophils show frank disruption with free granules (arrowheads).

In controls, only slight damage, ie, epithelial shedding of the outermost one or two layers in the anterior tip of the inferior turbinate (three cases) and slight intercellular edema (one case), was observed; no severe pathologic changes were observed.

For quantitative observation, three slices were randomly cut and the pathologic changes of the epithelium were quantitatively examined by light microscopy in both control and allergic specimens. A 5-mm length of epithelial lining was randomly examined in each slice. The total length of three slices was 15 mm of epithelial lining. Scoring was carried out so that + was one pathologic change, ++ was two pathologic changes, +++ was more than three pathologic changes, and – was no pathologic change in the 15-mm length of epithelial lining (Table 3).

In the anterior tip of the inferior turbinate of the allergy group, the epithelial shedding of the outermost one or two layers (slight epithelial shedding) and intercellular edema were seen in all patients, protrusion of the epithelium into the airway was observed in five cases (83%), and detached and destroyed epithelium (severe epithelial shedding) was present in three cases (50%; Table 3). Although, in the ciliated area (posterior) of the allergic group, slight epithelial shedding was observed in three cases (50%), intercellular edema was seen in four cases (67%), severe epithelial shedding was seen in one case (17%), and protrusion of the epithelium into the airway was not observed in any cases, and the grade was + in all positive cases. The frequency of observation of the pathologic changes of slight epithelial shedding, protrusion of epithelium into the airway, and intercellular edema was statistically (*t*-test) higher in the anterior area than in the posterior area (Fig 9), although the frequency of observation of severe epithelial shedding was statistically no different between the anterior and posterior areas (Fig 9). In the control group, slight epithelial shedding was observed in three cases (75%) in only the anterior area of the inferior turbinate, and intercellular edema was observed in one case (25%), in both the anterior and posterior areas, but epithelial protrusion into the airway and severe epithelial shedding were not observed in either the anterior or posterior area of the inferior turbinate. The grade was + in all positive cases. The frequency of observation of the pathologic changes of slight epithelial shedding, epithelial protrusion into the airway, and intercellular edema was statistically higher in the allergy group than in the control group, for the anterior area (Fig 9), but no significant difference was observed between the allergy group and the control group in the posterior area. The frequency of observation of severe epithelial shedding was statistically not different be-

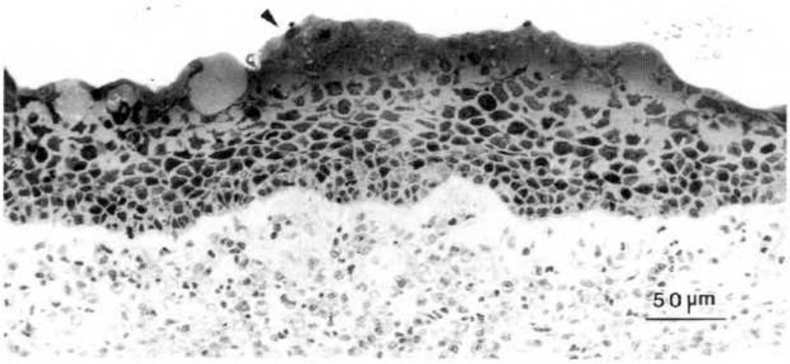

Fig 3. Edema is observed in intercellular spaces of anterior tip of inferior turbinate. Several outermost epithelial layers that protrude into airway lumen have degenerated (arrowhead).

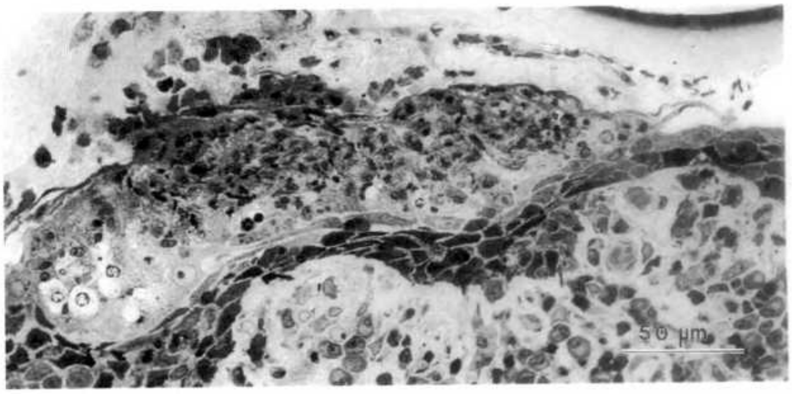

Fig 4. Most damaged epithelium of anterior tip of inferior turbinate. Epithelial cells have been shed and destroyed.

tween the allergy and control groups (Fig 9).

DISCUSSION

The fact that patients with asthma shed epithelial cells into the airway lumen is well established. For many years it has been recognized that the sputum of patients with asthma contains clumps of epithelial cells (Creola bodies) and that the number of these cells increases substantially during exacerbation of asthma.[15] In 1978, Cutz et al[16] first reported the changes in bronchial mucosa in biopsies from two young children with asthma. They showed the ultrastructural changes of bronchial epithelium: degenerated epithelial cells, ciliated cells with cytoplasmic blebs, and abnormal cilia. They were unable to observe evidence of squamous metaplasia or detachment of epithelial lining in their biopsy samples, however, although they found epithelial shedding in the autopsy samples of the patients, who died during an asthmatic attack. In 1985, Laitinen et al[3] reported that epithelial shedding was present in the biopsy samples even in subjects suffering from mild asthma. Watanabe and Watanabe[6] reported in 1981 that biopsy samples show the epithelium of patients with nasal allergy to be shed following provocation with allergen. Conversely, however, it was more recently reported that epithelial shedding was not observed in the nasal mucosa of the inferior turbinate

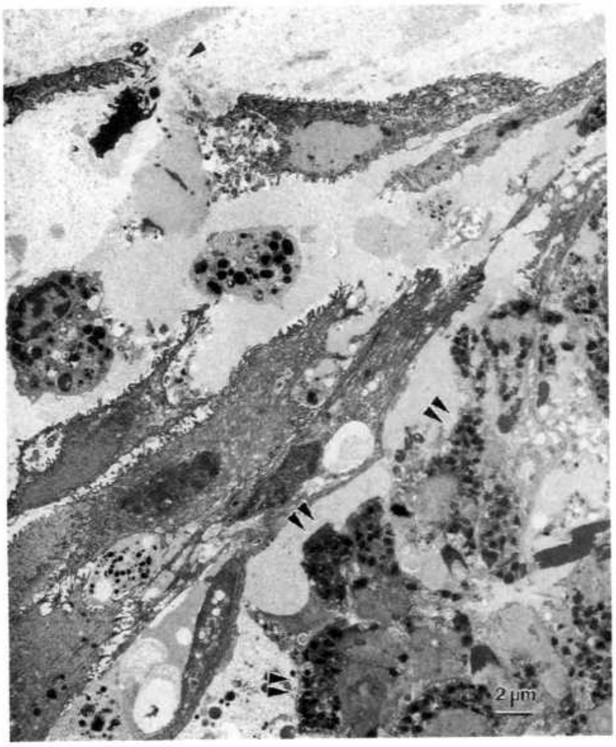

Fig 5. Electron microscopy showing severe damage to epithelium of anterior tip of inferior turbinate. Opened epithelial cell junction (arrowhead) and eosinophil cluster (double arrowheads) are observed.

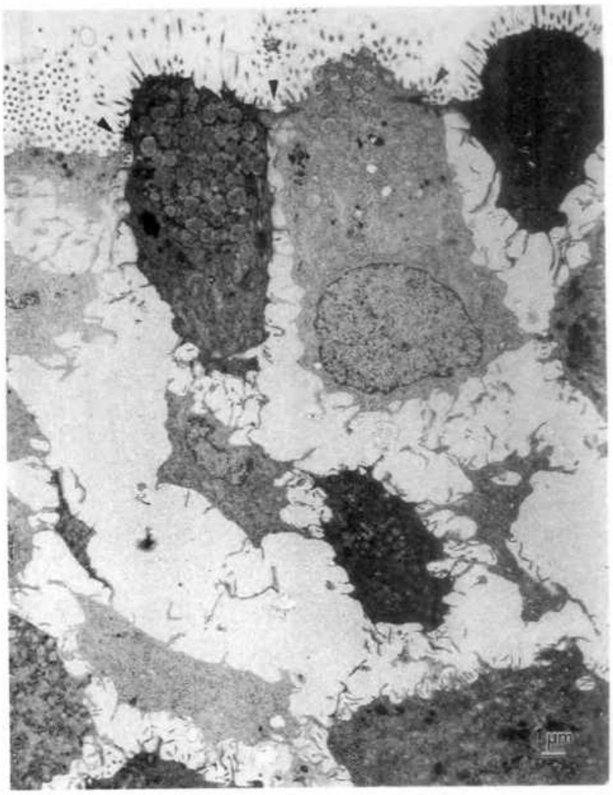

Fig 6. Epithelium in transitional zone from nonciliated area to ciliated area is composed of stratified columnar epithelial cells with microvilli. Intercellular space is wide, but epithelial cells remain adherent in tight junction (arrowheads).

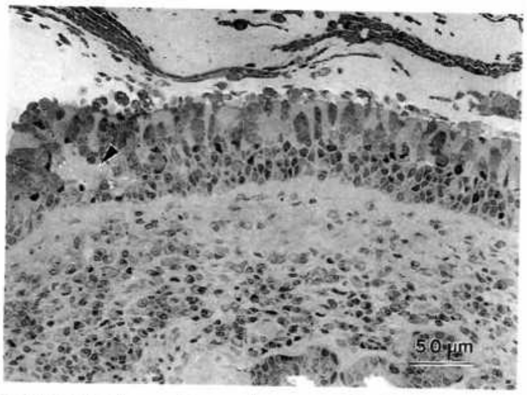

Fig 7. Epithelium of posterior area of inferior turbinate is composed of ciliated cells and goblet cells. Though epithelial shedding is not observed, edema (arrowhead) can be seen between basal cells and upper epithelial layers over wide area.

of patients afflicted with nasal allergy.[8] If the detachment of the epithelium was due to a mechanical separation consequent to contraction of the bronchial muscle,[17] the epithelial shedding would not occur in the nasal mucosa. However, it has been postulated that bronchospasm plays little or no part in the shedding of bronchial mucosa.[1] Barnes[5] advocated that patchy epithelial damage may be produced by basic proteins derived from eosinophils, perhaps also by the release of oxygen radicals from various inflammatory cells, and possibly as a consequence of submucosal edema. Since Gleich et al[18] reported the cytotoxicity of the eosinophil major basic protein in 1979, many researchers have showed that eosinophils cause epithelial injury to the tracheal mucosa.[2,7,19-25] Watanabe et al[26] reported in 1977 that eosinophil peroxidase, which is one of the proteins in the eosinophil granule, leaks out of the eosinophil and adheres to the nasal epithelium of nasal allergy patients. If such eosinophil granule proteins do indeed evoke such cytotoxic effects on tracheal epithelium as Gleich et al have reported, then they must generate the same effects on nasal epithelium. In the present study, many eosinophils were observed in the wide intercellular spaces between the nasal epithelial cells, with many of these eosinophils having migrated to the epithelial layer and sometimes forming clusters where the epithelial shedding was most conspicuous (Fig 5).

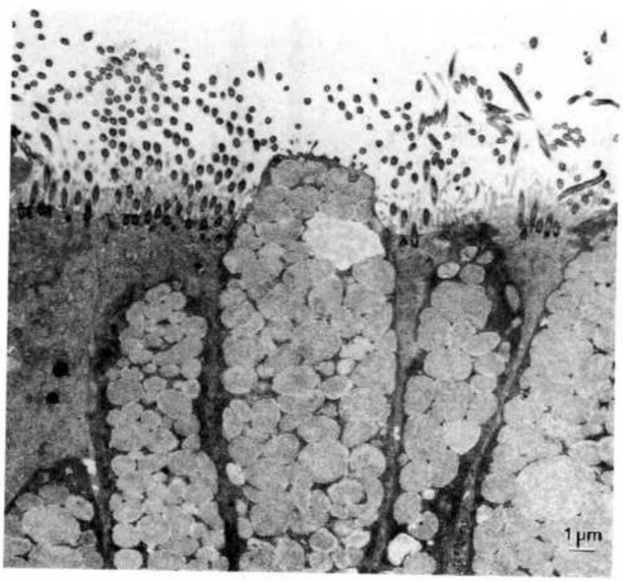

Fig 8. No intercellular edema is observed at surface of posterior turbinate by electron microscopy.

Barnes'[5] contention that edema of the epithelial intercellular spaces is also an important factor in epithelial shedding is reasonable. Dunnill[1] was the first to point out that in subjects who died of asthma, epithelial shedding appeared to be associated with submucosal and epithelial edema. Man et al[27] reported an interesting examination of intercellular edema

TABLE 3. QUANTITATIVE ANALYSIS

	Nasal Allergy Patients												*Controls*							
	Anterior Turbinate						*Posterior Turbinate*						*Anterior Turbinate*				*Posterior Turbinate*			
	1	*2*	*3*	*4*	*5*	*6*	*1*	*2*	*3*	*4*	*5*	*6*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*
Epithelial shedding of outermost one or two layers (slight epithelial shedding)	+++	+	++	++	+++	+++	–	+	–	+	+	–	+	+	–	+	–	–	–	–
Mean	2.33 ± 0.82						0.50 ± 0.55						0.75 ± 0.50				0			
Protruding of epithelium into airway	–	+	+++	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Mean	1.17 ± 0.98						0						0				0			
Intercellular edema	++	+++	+++	+++	+++	+++	–	+	+	–	+	+	–	+	–	–	–	–	+	–
Mean	2.83 ± 0.41						0.67 ± 0.52						0.25 ± 0.50				0.25 ± 0.50			
Detached and destroyed epithelium (severe epithelial shedding)	+	–	–	–	++	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–
Mean	0.67 ± 0.82						0.17 ± 0.41						0				0			

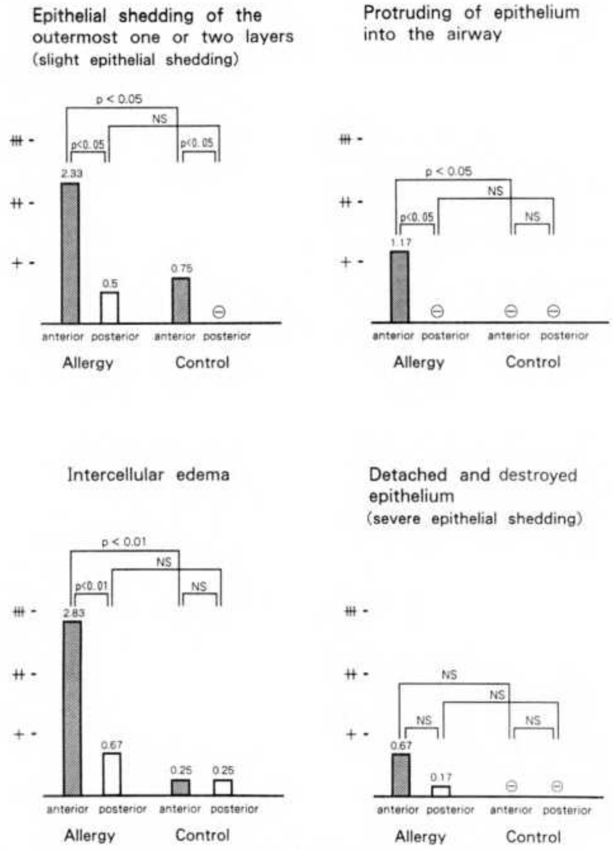

Fig 9. Statistical comparisons of nasal pathologic changes. Θ — no pathologic changes, + — one pathologic change, ++ — two pathologic changes, +++ — more than two pathologic changes, NS — not significant.

forming between epithelial cells; some of the epithelial cells shrank when an osmotic load was placed on the submucosal surface, but did not shrink when it was added to the mucosal fluid. Hogg and Walker[28] speculated that a large amount of fluid and protein leaked into the epithelial intercellular space and that if the solute were restricted at the epithelial surface and the fluid moved freely into the airway lumen, a submucosal osmotic load would be created and intercellular edema would be formed, as shown by Man et al. However, it is difficult to determine whether the edema fluid is secondary to the destruction of the bonds holding the epithelial cells together, or whether the edema fluid causes the separation of the epithelium.

In the present study, epithelial intercellular edema was observed to different degrees. It was frequently observed in the anterior nasal mucosae of the allergy group, but it was rarely seen in the posterior nasal mucosae, where ciliated and goblet cells existed. Shedding of the epithelial cells was also observed in the nonciliated rather than in the ciliated area. Epithelial intercellular edema is therefore considered one of the important factors in epithelial shedding. Although severe epithelial shedding (detached and destroyed epithelium) was not statistically different between allergy patients and controls, or between anterior and posterior nasal mucosae of allergy patients (Fig 9), the anterior nasal mucosae of allergy patients showed a marked tendency toward epithelial shedding. As most of the inhaled allergens are deposited in the anterior nasal mucosae,[10,14] the epithelial damage is assumed to be more severe in the anterior than in the posterior area. The basophils and mast cells (basophiloid cells) are also important cells in early and late phase reactions of allergy. In a previous paper,[29] we reported that the distribution of basophiloid cells in epithelial and shallow subepithelial layers was not statistically different between nonciliated and ciliated areas. It is considered that further examination is needed to clarify whether the basophiloid cells directly cause the epithelial damage or not, as we did not discuss the difference in degranulation rate of basophiloid cells between both areas in the previous paper. In this study, slight epithelial shedding was observed in the anterior nasal mucosa in three cases (75%), some of which were in the control group, although this pathologic change was observed with low frequency. This result means that the anterior tip of the inferior turbinate has slightly damaged epithelium, caused not only by allergen, but also by stimuli in unphysiological ambient air. Wihl and Mygind[9] reported that they could not discover any effect of intranasal challenge of allergen on ciliary function or epithelial structures, but they did not observe the anterior nasal mucosa. We think many researchers may have discussed nasal epithelial damage in the ciliated area but not in the anterior nasal mucosa. We consider that the anterior nasal mucosa is the most important and damageable portion in terms of nasal allergy.

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TRACHEAL RECONSTRUCTION WITH A VASCULARIZED CARTILAGE FLAP IN RABBITS

N. TAN ERGİN, MD
CAN KOÇ, MD
BEYHAN DEMİRHAN, MD
TEOMAN DAL, MD
ANKARA, TURKEY

The relative merits of a vascularized autogenous cartilage flap (VACF) are discussed in reconstruction of tracheal defects in rabbits. Sixteen albino Vienna rabbits were divided into two groups of eight animals each. In all rabbits, a rectangular tracheal defect was created. In the experimental group, a VACF was designed on the left auricle. This island flap was then transposed through a subcutaneous tunnel to the trachea and used to repair the defect. In the control group, tracheal defects were reconstructed with free cartilage grafts. In histologic evaluations, it was found that the reconstructed site showed a greater degree of vascularization when a VACF was used, and the gap between the trachea and flap was filled with new cartilage tissue, to a much greater extent than with the free cartilage grafts. It is concluded that use of the VACF is a relatively safe and effective procedure for repairing tracheal defects.

KEY WORDS — animal study, tracheal reconstruction, vascularized cartilage flap.

INTRODUCTION

Tracheal reconstruction is necessary in the management of stenoses and other laryngotracheal disorders. Although many innovative reconstructive techniques have been described, the most promising results have been obtained when autologous substitutes are used.[1-4]

The successful placement of a cartilage graft or flap, to provide a suitable tracheal function, requires that the following criteria be met[1]: 1) the tracheal surface must be thoroughly lined, 2) there should be a stable and structural framework, and 3) there must be adequate vascularization to keep the reconstructing component viable.

Despite advances of ingenious flap techniques, many difficulties remain in the reconstruction of tracheal defects. Although different success rates are reported with the application of myoperiosteal-myoosseous and myocutaneous flaps, many failures and high complication rates are also cited.[3,5-8] Absence of vascularization appears to play an important role in the failure of tracheal repair. In some studies, vascularized tissues have been used for revascularization of the reconstruction site.[1,4,6]

We herein present an experimental study in which a vascularized cartilage flap and a free cartilage graft were used to repair tracheal defects in rabbits, and the results of both types of repairs were compared histologically. To our knowledge, this is the first experimental documentation of tracheal reconstruction with a vascularized cartilage flap. With respect to the supporting value, the vascularized cartilage flap was superior to the cartilage grafts, and fulfilled the above-mentioned requirements.

MATERIALS AND METHODS

Sixteen albino Vienna rabbits weighing 2,000 to 2,500 g were divided into two groups and housed in University-approved quarters and fed with a standard rabbit diet. The experimental protocol was approved by the Baskent University Internal Animal Care and Use Committee. In the first group, tracheal defects were reconstructed with free cartilage grafts, while in the second group, vascularized cartilage grafts were used.

Operative Technique. The operative technique was identical in the two groups, except for the cartilaginous support. Anesthesia was obtained by intramuscular injection of xylazine hydrochloride (Rompun) 10 mg/kg and of ketamine hydrochloride 30 mg/kg. All animals received 180,000 units of penicillin G benzathine and penicillin G procaine intramuscularly preoperatively. The left auricle and neck of the animal were then shaved and prepared with povidone iodine. The main neurovascular bundle was injected at the base of the left ear with 1% lidocaine hydrochloride without epinephrine to provide additional anesthesia to the operative field.

A midline incision was made over the trachea, and the dissection was continued until the trachea was fully mobilized. A two-thirds resection of the six car-

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From the Departments of Otolaryngology (Ergin, Dal) and Pathology (Demirhan), Baskent University College of Medicine, and the Second Otolaryngology Clinic, Numune Hospital (Koç), Ankara, Turkey.
CORRESPONDENCE — N. Tan Ergin, MD, Baskent Üniversitesi KBB Anabilim dalı, 12 sokak 7/2, 06490 Bahçelievler, Ankara, Turkey.

COMPARISON OF TRACHEAL RECONSTRUCTION RESULTS

		Necrosis				
	Inflammation	*Minimal*	*Medium*	*Large*	*No Necrosis*	*Epithelization*
Free graft (n = 8)	3	0	4	4	0	4
Vascularized flap (n = 7)	0	3	1	0	3	7

tilaginous rings between the second and ninth tracheal rings was carried out. The left ear was brought into the sterile field, and a superficial incision was made through the skin, care being taken not to damage the perichondrial membrane. The skin was bluntly dissected on each side of the cartilage. In the first group, a free cartilage graft with its perichondrium on each side, 3 × 1 cm in diameter, was harvested. In the second group, the vascularized cartilage flap, 5 × 2 cm in diameter, with its axial pattern of caudal auricular vessels, was raised. A flap with a length of 5 cm and a width of 2 cm was obtained. This island flap was transposed through a subcutaneous tunnel to the trachea. Either the graft or the flap was sutured to the tracheal defect with interrupted nylon sutures. Gentian violet plus chloramphenicol ointment was lightly applied to the surgical wounds at the end of each procedure. Daily clinical evaluation was performed with particular attention to the animals' respiratory condition and local healing postoperatively.

The rabbits were painlessly sacrificed 35 to 42 days after surgery, and their tracheas were removed and examined both macroscopically and microscopically. The specimens were fixed in 4% neutral formalin and embedded in paraffin. They were sectioned and stained with hematoxylin and eosin, Alcian blue, and elastin dyes.

RESULTS

All eight rabbits in the free cartilage graft group and seven of the eight in the vascularized cartilage flap group survived. One of the rabbits in the vascularized cartilage flap group died on the 18th day due to problems secondary to an abscess that developed in the operative field.

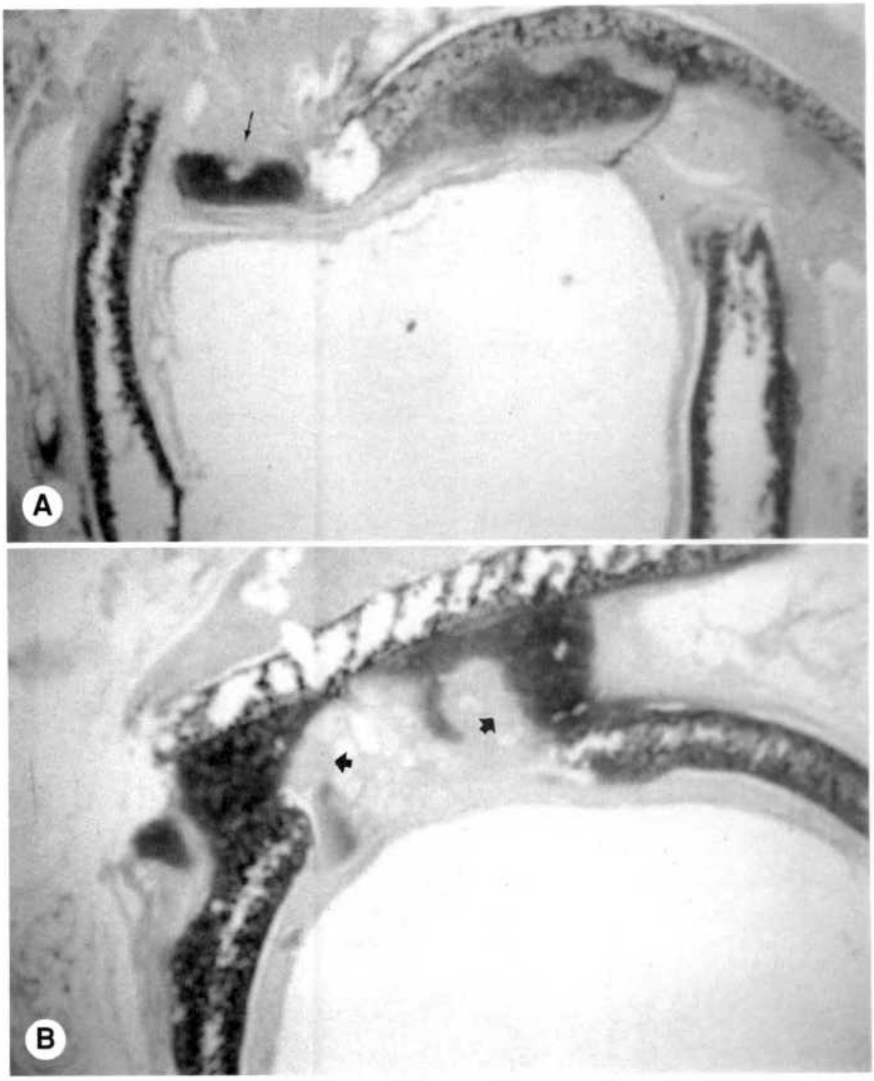
On macroscopic evaluation, no tracheal stenosis was evident in either group. The anteroposterior dimension of the reconstructed trachea was measured in all animals. In the vascularized cartilage flap group, the anteroposterior dimension of the tracheal lumens was normal (8 mm), whereas in the free cartilage graft group, only four lumens were normal. In the other four rabbits, the anteroposterior dimensions of the tracheal lumens were minimally (6 mm) narrowed; however, no respiratory obstruction was observed.

Histologic evaluation showed that the tracheal lumens were covered with epithelium in all specimens except for four rabbits in the free cartilage graft group (see Table). The perichondrium brought with the free or vascularized cartilage graft was able to provide a foundation for epithelization of the lumen. The vitality of the cartilage tissue was characterized by the appearance of nucleoli in chondrocytes, and the cells without nucleoli were accepted as necrotized. Necrotized cells were quantitatively scaled as follows: if there was necrosis in one microscopic area, it was called a minimal degree of necrosis; necrosis in two and more areas was called a medium degree of necrosis; and necrosis of a great part of the graft was called a large degree of necrosis. In the free cartilage graft group, there was medium necrosis in four rabbits and large necrosis in the other four; and in the vascularized cartilage graft group, there was minimal necrosis in three rabbits and medium necrosis in one rabbit, and no necrosis was observed in three specimens.

There was new cartilage tissue growth at the free border of the flaps in all specimens of the vascularized graft group and in four specimens of the free graft group (see Figure, A). New cartilage tissue growth is characterized by small and dense cells with little pericellular matrix. The gap between the trachea and graft was filled with fibroconnective tissue in general, but in three specimens from the vascularized cartilage graft group the gap was filled with new cartilage tissue (see Figure, B). Elastin dye–stained specimens demonstrated a relatively greater degree of vascularization in the vascularized cartilage graft group.

DISCUSSION

Surgical treatment of tracheal stenosis or defects may be simplified into endoscopic and open methods, based on the severity, extent, and location of the disorder. Open methods include excision of stenosis and stenting, use of various tracheal prostheses or alloplastic material, and a variety of techniques to augment the lumen.[7-9] These techniques use costal cartilage,[10] composite nasal septum cartilage,[11] a free periosteal graft,[12] a myoperiosteal flap,[13] a composite bone-muscle graft from hyoid[10,14] or clavicle,[15] and myocutaneous flaps such as the sternohyoid rotator door flaps[16] and the pectoralis major myocutaneous flap.[17] Results of such procedures frequently are unpredictable, and many failures and high



Histologic findings (Alcian blue, original ×27). A) New cartilage tissue (arrow) growth at free border of vascularized cartilage flap. B) Formation of new cartilage in gap between trachea and vascularized cartilage flap (arrows). In matrix, chondrocytes are seen, surrounded by defined capsules.

complication rates are cited, resulting in additional surgical treatment.[18,19] Reconstruction of a tracheal defect has been tried with prosthetic materials such as silicone rubber, porous mesh tubes, Teflon, and others.[7,9,20,21] The problems with all synthetic materials are the absence of an epithelialized surface, granulation tissue formation at the suture lines, and subsequent infection and extrusion. Cartilage autografts are preferable for restoring external support; they do not bear the risk of extrusion, as do alloplastic implants. Tracheal resection and end-to-end anastomosis appears to be indicated in patients with severe circumferential tracheal defects, and must be preserved as a last surgical procedure.

Consiglio[22] placed unlined grafts of auricular cartilage directly into tracheal windows both experimentally and clinically. Only atrophic epithelium was noted in some cases, but a fusion of the graft to the trachea and survival of the cartilage did occur. To extend this approach, Farmer et al[23] have also employed auricular cartilage in dogs, providing a mucous membrane covering by grafting a patch of buccal mucosa on the auricular perichondrium at an earlier stage. The composite graft was then transplanted into a tracheal window, with demonstrable survival and stability. On the other hand, Nakanishi et al[2] reported that tracheal autografts greater than 4 cm demonstrated malacia and necrosis of their midportion soon after transplantation in 25 dogs.

Experimental tracheal reconstruction with autotransplants has been studied by several investigators.[24,25] This type of free graft proved to be a far less than optimal procedure, since blood circulation to the donor grafts was not restored. In such donor grafts, there was no blood supply to the grafts other than the neovascularity that is passively derived from the recipient trachea during the course of healing. Revascularization is also useful in facilitating the healing of the tracheal reconstruction site; omentum, platysma myocutaneous flaps, and lateral thoracic fascia have been studied in this respect.[1-4,6,26,27] Clevens et al[28] used vascularized auricular perichondrium based on the central auricular artery and vein to reconstruct an anterior subglottic defect in New Zealand white rabbits.

A poorly vascularized area like the trachea has little revascularization potential and is prone to subsequent disintegration of an isolated tracheal autograft placed in the tracheal defect. The results of our study showed that a vascularized cartilage flap is not affected by the poor nutritional blood flow of the recipient tracheal defect; instead, the flap provides additional blood flow to enhance the wound repair process, thus minimizing the potential complications that have been reported when free cartilage grafts are used. Application of vascularized cartilage flaps in humans can be designed as a two-stage operation. In the first stage, septal or auricular cartilage is embedded in a highly vascularized area like the neck. In the second stage, cartilage with its vascular pedicle is transferred to the tracheal reconstruction site. Prefabrication of a vascularized cartilage flap that will be readily available for tracheal reconstruction should be studied further and might be promising for future clinical use.

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ROLE OF SUBSTANCE P IN THE LARYNGEAL CHEMOREFLEX

NANCY M. BAUMAN, MD
IOWA CITY, IOWA

DEQIANG WANG, MD
IOWA CITY, IOWA

DEBRA M. JAFFE, MD
IOWA CITY, IOWA

MICHAEL P. PORTER, MD
SEATTLE, WASHINGTON

TIMOTHY M. MCCULLOCH, MD
IOWA CITY, IOWA

RICHARD J. H. SMITH, MD
IOWA CITY, IOWA

ANTHONY D. SANDLER, MD
IOWA CITY, IOWA

The laryngeal chemoreflex (LCR) is a potentially life-threatening reflex that is elicited in immature animals by the topical application of water to the laryngeal mucosa. The reflex response is characterized by immediate apnea and laryngeal adduction and delayed cardiovascular instability. The cardiorespiratory changes of the LCR may be life-threatening, particularly in very immature animals such as piglets under 2 weeks of age. The afferent and efferent limbs of the LCR are mediated through the vagus nerve, but the neuromediators responsible for the reflex changes have not yet been clearly elucidated. Previous agonist and antagonist studies in immature dogs demonstrated that substance P, a sensory tachykinin, mediates the life-threatening esophagolaryngeal adductor reflex elicited by distal esophageal sensory nerve stimulation. This study was conducted to determine if substance P also plays a role in mediating the LCR. The LCR response was compared before and after treatment with intravenous substance P antagonist (Pfizer CP-96,345-1) in eight piglets (mean 27.7 days of age). The laryngeal and cardiovascular responses of the animals following intravenous administration of the tachykinins substance P, neurokinin A, and neurokinin B were also assessed. Pretreatment with substance P antagonist did not alter the LCR's duration of apnea ($p > .10$), laryngeal adductor response, or early change in mean arterial pressure ($p > .10$), although the early maximal heart rate response was significantly altered ($p < .01$). Intravenous substance P, neurokinin A, and neurokinin B did not reproduce the laryngeal respiratory response of the LCR. We conclude that substance P, neurokinin A, and neurokinin B are not key neurotransmitters of the LCR.

KEY WORDS — laryngeal chemoreflex, neurokinin, reflex, substance P, tachykinin.

INTRODUCTION

The laryngeal chemoreflex (LCR) is elicited by stimulation of afferent neurons of the laryngeal mucosa of many immature animals, including piglets, lambs, and kittens.[1-3] The LCR response is characterized by immediate apnea, laryngeal adduction, hypotension and bradycardia with rebound hypertension, and tachycardia. Reflex laryngeal adduction may be excessive and induce laryngospasm, particularly in immature animals. The reflex response is implicated as a potential cause of sudden infant death syndrome (SIDS) and apparent life-threatening events (ALTEs), although, to date, it has only been elucidated in laboratory studies.[4] The afferent and efferent limbs of the LCR are mediated through vagal nerve branches. Although the reflex response is diminished by intrathecal atropine[5] and by aerosolized or intravenous diphenhydramine,[6,7] the neurotransmitters responsible for conducting the LCR remain to be elucidated.

Previous studies in our laboratory identified another reflex response in immature dogs in which laryngeal adduction is also a key feature. This response, termed the esophagolaryngeal adductor reflex (ELAR), is elicited by stimulation of afferent sensory neurons of the distal esophagus by the topical application of capsaicin (8-methyl-N-vanillyl-6-nonenamide).[8] The response is characterized by intense laryngeal adduction, tachycardia, and hypertension. Studies completed in our laboratory utilizing intravenous infusions of substance P (SP) and antagonists demonstrated that SP is an essential neurotransmitter of the canine ELAR (also A. D. Sandler et al, unpublished data, 1994).

The ELAR and LCR share important characteristic features, including life-threatening laryngeal adduction, cardiovascular instability, and a predilection to occur in immature animals. These similarities, as well as the recognized role of SP as a primary sensory neurotransmitter, prompted the present

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From the Pediatric Division of the Department of Otolaryngology–Head and Neck Surgery (Bauman, Smith), the Department of Otolaryngology–Head and Neck Surgery (Wang, Jaffe, McCulloch, Porter), and the Pediatric Division of the Department of General Surgery (Sandler), The University of Iowa College of Medicine, Iowa City, Iowa. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of The University of Iowa.

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CORRESPONDENCE — Nancy M. Bauman, MD, Dept of Otolaryngology–Head and Neck Surgery, Pediatric Division, The University of Iowa College of Medicine, Iowa City, IA 52242.

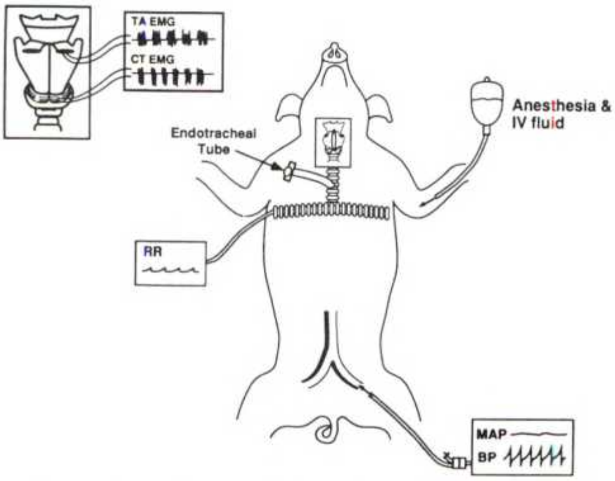

Fig 1. Animal preparation. Systemic blood pressure (BP), mean arterial pressure (MAP), heart rate, respiratory rate (RR), and cricothyroid (CT EMG) and thyroarytenoid electromyograms (TA EMG) were monitored in piglets. Laryngeal test fluids were applied via midline thyrotomy. IV — intravenous.

study to determine if SP plays a significant role in eliciting the LCR.

MATERIALS AND METHODS

Preparation. Nine mixed-breed piglets were anesthetized with intramuscular ketamine with 5% xylazine (35 mg/kg). Intravenous access was established, and one third of the initial dose was subsequently administered intravenously every 20 to 30 minutes to maintain an adequate depth of anesthesia as determined by the presence of spontaneous respirations, absence of purposeful movement, and absence of corneal reflexes.

The larynx and superior trachea were exposed through a horizontal cervical incision (Fig 1). An endotracheal tube was placed through the fourth tracheal ring, and the animal was mechanically ventilated for the remainder of the surgical preparation period. The laryngeal mucosa and vocal folds were exposed through a midline thyrotomy. Bipolar, hooked wire, stainless steel electrodes were placed unilaterally into the cricothyroid (CT) and thyroarytenoid (TA) muscles under direct vision. The electrodes were connected to the voltage pulse input coupler of the recorder, and the signal was amplified, digitized, and monitored on-line. Electrode placement was verified by the phasic, audible electromyography (EMG) signal in relationship to the respiratory cycle. Activity was recorded on a computer hard drive (Gateway 2000, 4DX2-66V) with Codas Windaq software (Akron, Ohio) and viewed on-line on a computer terminal (Gateway 2000, Crystal Scan). Information was transferred to a magnetic optical disk for subsequent review. Once positioned, the electrodes were not moved during the course of the study.

Systemic blood pressure and heart rate (HR) were measured via a femoral catheter line connected to an external pressure transducer. Respiratory rate (RR) was measured by a belt pneumotachogram. These amplified signals were also monitored on-line, recorded, and transferred to a magnetic optical disk.

Physiologic body temperature was maintained with a warming blanket. All procedures were approved by the institutional Animal Research Committee and were in accordance with guidelines set forth by the National Institutes of Health.

Eliciting Laryngeal Chemoreflex. Mechanical ventilation was terminated and the animals breathed spontaneously through the endotracheal tube cannulating the distal (inferior) trachea.

Two milliliters of distilled water (test fluid) were gently applied to the laryngeal mucosa via a syringe and catheter following a control application of saline. Test fluids were not applied until 10 to 15 minutes had elapsed after the previous dose of intravenous anesthesia. Adequate time was permitted for return to baseline cardiovascular and respiratory parameters between test fluid applications (>10 minutes). Following fluid application, the vocal folds were observed for laryngeal adduction. Cardiovascular, respiratory, and laryngeal adductor EMG responses were monitored, and baseline and peak responses recorded. Arterial blood gases (ABGs) were obtained during baseline periods and at the point of maximal decrease in mean arterial pressure (MAP) induced by water application. The procedure was repeated once a minimum of 10 minutes had elapsed after the hemodynamic parameters returned to baseline. An average "baseline LCR response" for each animal was established.

Substance P, Neurokinin A, and Neurokinin B Infusions. Cardiovascular, respiratory, and laryngeal EMG responses were recorded before and after intravenous infusion of 1 µg/kg of SP (Sigma Chemical) to assess the role of SP in the LCR. The SP was diluted in 1% human serum albumin in saline and stored at –20°C prior to administration. The dose utilized was derived from previous studies determining the minimal effective dose for inducing the maximal change in lower esophageal smooth muscle contraction.[9] Cardiovascular, respiratory, and laryngeal EMG responses were also recorded after intravenous infusion of the related tachykinins neurokinin A (NKA) and neurokinin B (NKB). Both NKA and NKB were previously studied in dogs and found not to play a significant role in the ELAR (A. D. Sandler et al, unpublished data, 1994).

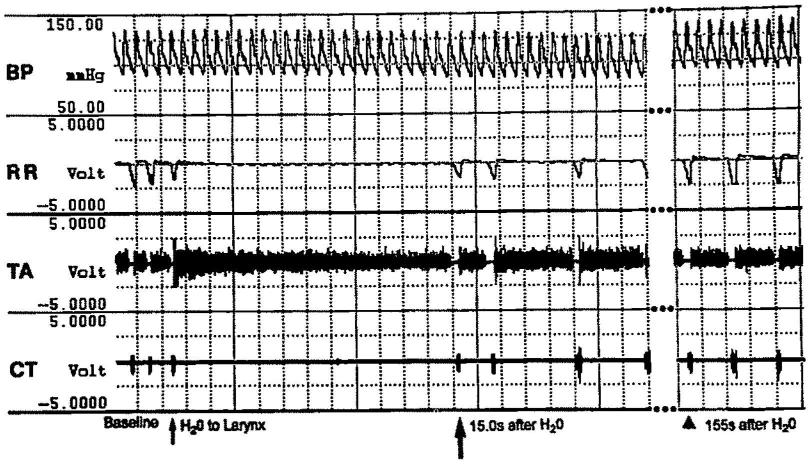

Fig 2. Piglet laryngeal chemoreflex (LCR). Characteristic cardiovascular (BP), respiratory (RR), TA and CT EMG responses of LCR demonstrated in 47-day-old piglet. Each square represents 1.25 seconds. Note immediate central apnea of 15.0 seconds' duration and early decrease in BP (large arrow) following water application to laryngeal mucosa (small arrow). CT activity increased slightly after apnea response (triangle), and accompanied increased depth of respiratory excursion. Delayed rebound hypertension was also noted (triangle). Codas Windaq software.

Substance P Antagonist Study. Animals were pretreated intravenously with 1 mg/kg of an NK1-specific SP antagonist (Pfizer CP-96,345-1). Previous studies confirmed this intravenous dose to be effective in blocking the ELAR (A. D. Sandler et al, unpublished data, 1994). Water was subsequently applied to the laryngeal mucosa, and parameters were recorded for comparison with the baseline LCR response to determine the effect of the SP antagonist infusion.

DATA ANALYSIS

Laryngeal Chemoreflex. Baseline (prestimulus) respiratory, laryngeal, and cardiovascular parameters were compared with peak values obtained after application of water to the laryngeal mucosa. Each animal served as its own control and underwent 2 to 5 water applications. Apnea was defined as cessation of breathing and measured in seconds from the end of expiration to the beginning of the next regular inspiration. Numerical data on MAP, HR, and RR were calculated by Windaq software. The MAP and HR values were determined by averaging three consecutive waves, and RR by averaging four consecutive waves. Minimum and maximum values established for each animal were averaged by time sequence to eliminate the variable response time between animals. Cardiovascular and respiratory responses were compared between the animals by using Student's *t*-test, with $p < .05$ accepted as statistically significant. Association of responses was analyzed by regression analysis. Water applications were excluded from analysis if characteristic cardiovascular changes did not accompany the apneic response, as these were not considered "true" LCR responses.

Substance P, Neurokinin A, and Neurokinin B. Respiratory and laryngeal parameters after SP, NKA, and NKB infusions were compared to the parameters of the LCR response. Cardiovascular changes were not compared, as the tachykinins often induce direct vasodilation and resultant hypotension.

Substance P Antagonist. The effect of SP antagonist on the LCR was assessed in each animal by comparing the length of apnea and the percent change of the maximum and minimum cardiovascular and respiratory parameters of the LCR before and after pretreatment with SP antagonist.

RESULTS

Laryngeal Chemoreflex. The LCR was elicited in nine piglets between 14 and 47 days of age (mean 27.7 days). The animals' mean weight was 7.3 kg (range 5.3 to 10.1). One piglet succumbed to cardiovascular collapse following LCR-induced apnea of 58 seconds. The eight surviving piglets underwent the SP antagonist and SP agonist studies and constituted the study group on which the following results are reported.

The LCR was elicited an average of 2.5 times per animal prior to, and 2.8 times per animal after, pretreatment with SP antagonist. Adequate time elapsed between water applications to guard against a waning response. Two of 46 responses were excluded from analysis, as the characteristic cardiovascular changes of the LCR did not accompany the apneic response.

Respiratory Response. Saline applied to the mucosa of the larynx did not elicit apnea, although some animals experienced mild irregularity of their respiratory cycle, likely due to mechanoreceptor stimulation. Water application elicited immediate central apnea in all animals that lasted a mean of 13.2 seconds (Fig 2). The duration of apnea varied between animals, but, with few exceptions, remained relatively constant for each animal. The mean duration of apnea was longest in those piglets under 3 weeks of age, and the only piglet to experience a fatal response was 17 days of age.

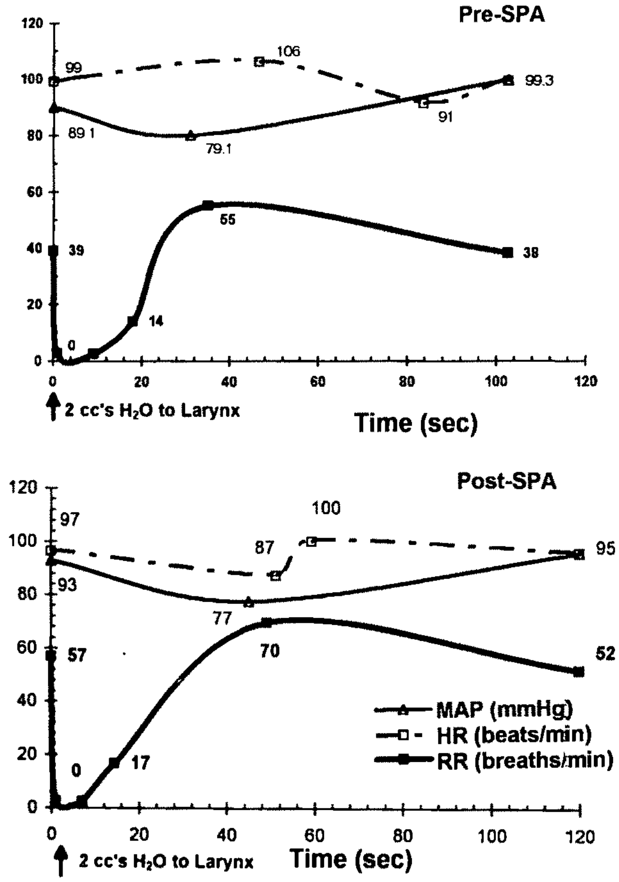

Fig 3. Mean LCR responses in eight piglets, 17 to 47 days of age, elicited by laryngeal water application and adjusted for mean time to peak responses (upper graph). Note early cessation of respirations (RR), early decrease in mean arterial pressure (MAP), and delayed increase in heart rate (HR). Lower graph shows mean LCR responses in same piglets following pretreatment with substance P antagonist (SPA). Note similar respiratory rate and MAP changes but opposing HR responses.

Room air ABGs were analyzed before and after water application in six of the piglets. Pre-LCR blood samples were obtained immediately prior to water application, and post-LCR blood samples were obtained when the MAP was at its nadir, usually within 1 minute of the water application. The arterial oxygen concentration tended to decrease during the LCR, and the carbon dioxide concentration tended to increase, although neither change was statistically significant ($p > .05$). These trends were attributed to the central apnea and not the laryngeal adduction, as the animals' distal tracheas remained cannulated throughout the studies.

Laryngeal Electromyography Response. During resting respiration, the TA muscle fired at the onset of expiratory chest wall recoil, while the CT muscle fired during inspiratory chest wall expansion (Fig 2). The CT EMG signal was consistently smaller than the TA signal in piglets, and although an audible CT EMG signal was usually present, it often did not generate a visible waveform on the monitor during resting (baseline) respiration.

Saline applied to the laryngeal mucosa did not alter laryngeal adductor activity. Immediately following water application, the TA muscle fired continuously and the vocal folds were visibly adducted in laryngospasm. The apnea was thus of a mixed central and obstructive pattern, with the central component persisting a mean of 1.2 seconds longer than the obstructive component (Fig 2).

The magnitude of phasic CT muscle activity increased slightly following water application, but the response was delayed and did not occur until the mixed apnea was over (Fig 2). Although voltage changes were not calculated, the slight increase in CT activity appeared to accompany an increased depth of respiration.

Cardiovascular Responses. The MAP decreased significantly following water application, from an average of 89.1 mm Hg to an average of 79.1 mm Hg ($p < .01$; Fig 3, upper graph). The time to this peak response varied between animals and occurred a mean of 32 seconds after the water stimulus. The MAP returned to baseline and subsequently increased to 99.3 mm Hg ($p < .01$).

Early changes in HR during apnea were variable. Some animals experienced initial increases, while others experienced minimal changes. The mean HR for all eight animals, however, increased significantly, from 99 beats per minute to 106 beats per minute ($p < .01$; Fig 3). This change lagged behind the decrease in MAP and occurred a mean of 48 seconds after water application. The increase in HR did not correlate well with animal age ($r = -.25$), weight ($r = .28$), degree of apnea ($r = .55$), or initial decrease in MAP ($r = -.47$). As the MAP subsequently increased, the HR tended to decrease, although not significantly ($p > .05$; Fig 3, upper graph).

Response to Substance P Infusion. The effect of SP intravenous infusion was also assessed in eight piglets (14 to 47 days of age). The MAP significantly decreased, reaching a nadir 20.8 seconds after infusion that was attributed to SP's known vasodilatory effect. The TA and CT phasic respiratory activities slightly increased 43.6 seconds (mean) after infusion. Five animals experienced brief periods of central apnea lasting an average of 9.5 seconds, but the fact that the apnea did not begin until a mean of 102 seconds (range 52 to 300) after the infusion suggests that the response was more likely secondary to concurrent and/or preceding cardiovascular changes.

Laryngeal Chemoreflex Response Following Pre-

PIGLET LCR RESPONSE BEFORE AND AFTER ADMINISTRATION OF SP ANTAGONIST (N = 8)

Parameter	*Pre-SP Antagonist LCR*	*Post-SP Antagonist LCR*	*Pre vs Post*
Apnea	13.2 seconds	11.6 seconds	p > .10
MAP	↓ then ↑	↓ then ↑	p > .10
Early HR	↑ then ↓	↓ then ↑	p < .01
TA EMG	↑↑ immediate	↑↑ immediate	No visible difference*
CT EMG	↑ delayed	↑ delayed	No visible difference*

LCR — laryngeal chemoreflex, SP — substance P, MAP — mean arterial pressure, HR — heart rate, TA — thyroarytenoid, EMG — electromyography, CT — cricothyroid.

*Voltage not measured; p value not determined.

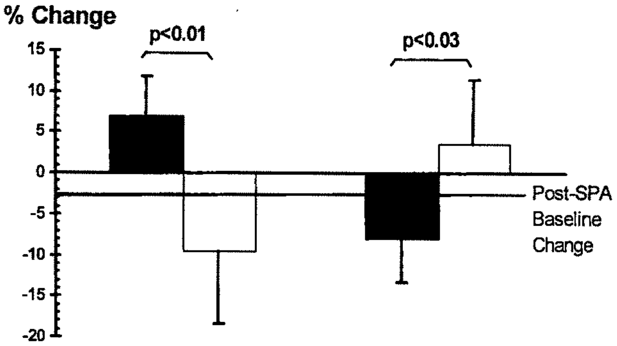

Fig 4. Early (left) and delayed (right) HR response of piglet LCR before (black) and after (white) treatment with SPA (n = 8). Note significant differences in HR response.

treatment With Substance P Antagonist. Substance P antagonist was infused in the same eight piglets. The baseline respiratory rate was not altered by infusion of the SP antagonist (p > .05).

The LCR was elicited when cardiorespiratory parameters had returned to baseline values, in order to further determine the role of SP in the LCR. Compared to the "baseline" LCR, no significant change in apnea duration (p > .10), visible CT and TA EMG activity, or early mean decrease in MAP (p > .10) was noted (Fig 3, lower graph, and Table). Piglets did experience an early mean decrease in HR during the LCR response, contrary to the early mean increase in HR noted with the LCR prior to pretreatment with SP antagonist (p < .01; Figs 3 and 4). The direction (increase versus decrease) of the delayed HR response of the LCR also reversed following pretreatment with SP antagonist (p < .03; Fig 4).

Response to Neurokinin A and Neurokinin B Infusions. Neurokinin A and NKB were intravenously infused in three piglets between 17 and 29 days of age. A decrease in MAP followed the NKA infusion, but significant changes in adductor activity or RR did not occur. The MAP, adductor activity, and RR remained unchanged following infusion of NKB.

DISCUSSION

Excessive or inappropriate laryngeal reflex responses have been implicated as a mechanism of ALTEs and SIDS.[10] Intense laryngeal adduction is a component of both the LCR and the ELAR, and although these reflexes have only been studied in animal models, they are thought to play a role in infant morbidity and mortality. Both reflexes are most pronounced in immature animals and are mediated by the vagus nerve[11] (also A. D. Sandler et al, unpublished data, 1994). Despite these similarities, the role of SP differs markedly between the ELAR and the LCR. While previous intravenous agonist and antagonist studies established a role for SP as a key neurotransmitter of the ELAR (A. D. Sandler et al, unpublished data, 1994), SP does not play a significant role in mediating the LCR. In this study, SP infusion did not mimic the laryngeal response of the LCR, and pretreatment with SP antagonist did not significantly alter the key components of the LCR.

Pretreatment with SP antagonist did alter the HR response of the LCR, but we believe that this most likely reflected an effect on the chemoreceptor and/or baroreceptor regulatory systems. Although the mean HR of the piglets increased when the LCR was elicited, the response of individual animals was variable and only occurred after significant changes in respiration and blood pressure occurred. This temporal pattern suggests that the changes in HR occur as a compensatory component of the LCR reflecting a complex interaction between the chemoreceptor system (activated by changes in blood gas concentration tending to decrease HR) and baroreceptors (activated by decreased MAP tending to increase HR to maintain cardiac output). The mean HR increased during the LCR, but decreased in animals pretreated with SP antagonist. We postulate that this change may be attributed to inhibition of chemoreceptor activation by the SP antagonist. Substance P has been identified in chemoreceptor tissue, and previous studies have demonstrated that pharmacologic antagonism reduces, in a dose-dependent fashion, the cardiovascular responses to hypoxemia.[12]

Neurokinin A and NKB infusions did not demonstrate laryngeal or cardiovascular changes suggestive of the LCR. These neurotransmitters appear to play no significant role in mediating the LCR.

SUMMARY

Increased laryngeal adductor activity is a key component of both the LCR and the ELAR, life-threatening reflexes present in immature animals. Despite several similarities of these reflexes, SP plays a key role in mediating the canine ELAR, but does not appear to play a significant role in the piglet LCR. Antagonism of SP receptors may, however, alter the compensatory cardiovascular responses of the LCR.

CONCLUSIONS

1. Substance P does not play a key role in mediating the characteristic laryngeal and respiratory responses of the LCR.

2. Pretreatment with SP antagonist alters the delayed cardiovascular responses of the LCR, perhaps by modulation of the compensatory changes induced by the arterial chemoreceptor system.

3. Neurokinin A and NKB infusions do not mimic the respiratory or laryngeal response of the LCR and do not likely play a significant role in mediating the reflex.

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RHINOLOGY SYMPOSIA

The 18th International Symposium on Infection and Allergy of the Nose (ISIAN), the 4th Asian Research Symposium on Rhinology (ARSR), and the 3rd ASEAN Rhinology Group Congress (ARC) will be held in Manila, Philippines, February 14-18, 1999. For information, contact Gil M. Vicente, MD, Congress President, Dept of ORL-HNS, Jose Reyes Memorial Medical Center, Rizal Avenue Sta. Cruz, Manila, Philippines 1003; telephone (632) 743-6921; fax (632) 732-1077 or (632) 522-0946; e-mail isian@psohns.com; web site http://www.psohns.com/isian.

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RECONSTRUCTION WITH FREE JEJUNAL AUTOGRAFT AFTER CIRCUMFERENTIAL PHARYNGOLARYNGECTOMY: EIGHTY-THREE CASES

MORBIZE JULIERON, MD
MICHEL A. GERMAIN, MD
GUY SCHWAAB, MD
PATRICK MARANDAS, MD
JEAN-LOUIS BOURGAIN, MD
PIERRE WIBAULT, MD
BERNARD LUBOINSKI, MD
VILLEJUIF, FRANCE

The free jejunal autograft (FJA) has become a common procedure for pharyngeal reconstruction after circumferential pharyngolaryngectomy. In order to evaluate the postoperative outcome and the functional and carcinologic results, we retrospectively reviewed 83 cases of reconstruction with FJA. Fifty-one patients had received no prior radiotherapy, and 25 had received prior radiotherapy for their hypopharyngeal tumor or for another previous primary. Seven patients underwent a secondary reconstruction. In the postoperative course, there were 2 postoperative deaths, 4 graft failures (5%), and 11 salivary fistulas. The median time to removal of the nasogastric tube was 16 days, and to discharge, 23 days. Forty-eight patients received postoperative radiotherapy, with good tolerance. At 1 year postoperatively, 98% of the patients were able to eat a solid or soft diet. The postoperative radiotherapy did not impair the quality of the functional results in a long-term assessment. The vocal results were disappointing. The 3-year survival rate was 40%. The main carcinologic failures (45 patients) were locoregional recurrences (20 patients) and metastasis, which was the cause of death in 34% of the cases. It seems clear that FJA allows one-stage reconstruction and good swallowing rehabilitation, tolerates postoperative radiotherapy, and increases the quality of life in these patients with a poor prognosis.

KEY WORDS — free jejunal autograft, hypopharyngeal tumors, total pharyngolaryngectomy with cervical esophagectomy.

INTRODUCTION

The free jejunal autograft (FJA) has become a common procedure for pharyngeal reconstruction after total pharyngolaryngectomy with cervical esophagectomy. The development of microsurgical techniques and the improvement of perioperative management have led to good reliability of this kind of reconstruction, and at present, the success rate of FJA is over 90% in almost all recent series, with very low rates of postoperative mortality and morbidity.[1-3] The aim of this reconstructive option is to allow one-stage reconstruction and early swallowing rehabilitation. Indeed, the prognosis of patients with widespread pharyngeal tumors is poor, with a high risk of locoregional recurrences and metastasis.[4] It is important for such patients to obtain early rehabilitation and a good quality of life. The quick rehabilitation of such patients is pointed out in many series, but the long-term functional outcome is seldom described, nor are carcinologic results. In order to explore these aspects, we retrospectively reviewed 83 cases of reconstruction of the pharynx with FJA.

PATIENTS AND METHODS

A total of 87 patients who underwent resection of head and neck tumors had the continuity of the upper digestive tract restored by FJA at the Institut Gustave Roussy (Villejuif, France) between January 1982 and September 1995 (90% were operated on after January 1989). In 4 patients, the reconstruction was performed after total pharyngolaryngectomy with total esophagectomy, because other attempted methods of reconstruction had been unsuccessful. The other 83 patients were reviewed. They ranged in age from 18 to 72 years (median 55 years). Seventy-five were men and 8 were women. Seventy-six patients underwent primary reconstruction after circumferential resection for hypopharyngeal carcinomas. Among them, 51 patients had received no prior radiotherapy. Twenty-five had received prior radiotherapy (14 for their hypopharyngeal tumor; 11 for another previous primary). Seven patients underwent secondary reconstructions after the failure of alternative reconstructive procedures following circumferential pharyngolaryngectomy (3 cases) or for wound healing or stricture after total laryngectomy with partial pharyngectomy (4 cases). Squamous cell carcinoma was the histologic diagnosis in all patients. The staging evaluation included laryngoscopy, esophagoscopy, computed tomography scans of the neck and thorax, and an ultrasound examination of

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From the Departments of Head and Neck Surgery (Julieron, Germain, Schwaab, Marandas, Luboinski), Anesthesiology (Bourgain), and Radiation Oncology (Wibault), Institut Gustave Roussy, Villejuif, France.

CORRESPONDENCE — Morbize Julieron, MD, Département d'ORL et de Chirurgie cervicofaciale, Institut Gustave Roussy, 39 rue C. Desmoulins 94800, Villejuif, France.

TABLE 1. TNM DISTRIBUTION

	N0	*N1*	*N2a*	*N2b*	*N2c*	*N3*	*Nx*	
T1								0
T2	1	2			3			6
T3	4	4	1		4	1		14
T4	19	8	1	2		1	1	32
Tx							27	27
Total	24	14	2	2	7	2	28	79

the liver. The TNM classification of this series is shown in Table 1. The primary tumor sites and extensions are shown in Table 2. A thorough assessment of pulmonary and cardiac status was carried out for all patients. The cervical vascular status was studied in 51 patients by using cervical Doppler to search for vascular atheroma.

Surgical Procedure. All procedures are performed with a two-team approach. While the head and neck team performs the resection, the second team performs the laparotomy and selects a jejunal segment with vascular anatomy suitable for preparation of a long mesenteric vascular pedicle. Usually, the third jejunal segment is the most convenient, with a constant and large pedicle. The segment is resected with preservation of the remnant jejunum vascularization. Transillumination is used to ascertain whether mesenteric arcades are sufficient to ensure the vascularization of this remnant jejunum. Then, an end-to-end digestive anastomosis is performed. The pedicle of the jejunal segment is cut only at the end of tumor resection and cervical recipient vessel dissection. The graft is usually 20 to 25 cm, which is always enough to repair the pharyngeal defect. In the meantime, the head and neck team performs tumoral resection and nodal dissection including upper mediastinal node dissection. Ten minutes before the jejunal pedicle is cut, anticoagulant therapy is begun. The isolated jejunum is inset in an isoperistaltic manner and cooled with a plastic bag containing ice during the anastomosis. We usually perform the vascular anastomosis first, to decrease the ischemic time of the graft. The anastomosis of the venous supply is done before the arterial supply. Usually, it is an end-to-side anastomosis with the internal jugular vein. The arterial anastomosis is usually performed with an external carotid branch. An anticoagulant is used to flush the vessels during microanastomosis. In the beginning of this series we used unfractionated heparin, and more recently, low–molecular weight heparins. Enteric sutures are then placed, starting with the oropharynx. The cephalad jejunum is opened along its antimesenteric border to match its size with the circumference of the oropharynx. Then, enteric sutures with the remnant esophagus are placed in two layers as interrupted sutures, and a nasogastric tube is inserted. Redundant mesentery is wrapped around the superior enteric suture to decrease the risk of postoperative fistula. During the first postoperative days, the state of the flap is submitted to clinical examination (direct examination or fiberscopic control). Monitoring is also performed twice a day through the use of external Doppler to control the patency of the artery and vein of the flap. Anticoagulant therapy is administered subcutaneously every 12 hours for 15 days. A barium swallow test is performed on the 9th day to ensure that there is no anastomosis leak. Oral nutrition is then allowed and the nasogastric tube is removed.

TABLE 2. SITES OF ORIGIN OF TUMORS AND EXTENSIONS IN 79 PATIENTS WHO UNDERWENT CIRCUMFERENTIAL RESECTION FOR TUMOR

	Site of Origin	*Extensions*
Pyriform sinus	40	36
Posterior pharyngeal wall	18	39
Retrocricoid area	6	24
Pharyngoesophageal junction	5	29
Multiple in hypopharynx	10	
Oropharynx		16
Cervical esophagus		3
Trachea		0

Adjuvant Radiotherapy and Chemotherapy. Excluding the 32 patients who had radiotherapy previously, 48 patients received postoperative radiotherapy; the dose was more than 60 Gy in 23 patients. Fifteen patients were given preoperative chemotherapy with cisplatin and 5-fluorouracil.

Follow-up was complete through the end of May 1996 in 81 patients. Death or complications occurring during the first 30 postoperative days were considered postoperative complications. We studied short-term functional results: time to removal of the nasogastric tube and time to the patient's discharge. Long-term functional assessment was done in patients free of recurrence at 6 months, 1 year postoperatively, and every following year. An evaluation scale was developed for swallowing (normal diet, soft diet, liquid diet, tube feeding) and speech rehabilitation (excellent, good, fair, poor). The χ^2 test was employed for statistical analysis. Carcinologic results were studied for the 79 patients who underwent circumferential resection of tumor (the 4 patients who underwent secondary reconstructions after total laryngectomy with partial pharyngectomy were excluded). Survival was calculated by the Kaplan-Meier method. Comparison of survival between the subgroups of patients was performed with the log rank test. A $p < .05$ was considered statistically significant.

TABLE 3. LOCAL AND GENERAL COMPLICATIONS

	Patients	
Complications	No.	%
General	20	24.1
Pulmonary	7	8.4
Sepsis	5	6.0
Decompensation of previous defect	3	3.6
Hypocalcemia	4	4.8
Other	7	8.4
Postoperative death	2	2.4
Local	33	39.8
Graft failure	4	4.8
Salivary fistula	11	13.3
Cervical abscess	7	8.4
Hematoma	7	8.4
Other	9	10.9

RESULTS

Postoperative Course and Short-Term Functional Results. In the postoperative course 20 patients had general complications, and 33, local complications. Two postoperative deaths occurred: 1 due to bleeding and perforation of a gastric ulcer, another to a carotid hemorrhage due to a salivary fistula in an irradiated area. The details of these complications are given in Table 3. Four graft failures occurred (4.8%), but in 1 of these cases the FJA was immediately replaced by another one with success. The other 3 patients underwent reconstructions with alternative methods using pedicled myocutaneous flaps. Two of these graft failures occurred in previously irradiated areas (2/32). The overall success rate of the microvascular technique was thus 96.4%. Eleven salivary fistulas occurred, 10 of which were clinically detected; 1 was only visible on the barium swallow test. Of those fistulas, 6 occurred on a previously irradiated bed (6/32). Among the 11 fistulas, 3 required a reoperation with the insetting of a myocutaneous flap to cover cervical vessels; 5 closed spontaneously; 3 were not repaired because of recurrent tumor or the patient's death.

The nasogastric tube was removed before the 16th day in more than half of the patients (mean 28 days, range 8 to 360). The median time to patients' discharge was 23 days (mean 27, range 11 to 130). In patients who had previous radiotherapy, the median time for the removal of the nasogastric tube was 16 days (mean 48, range 9 to 360) and the time to discharge was 21 days (mean 28, range 11 to 83). Among the 48 patients who received postoperative radiotherapy, the tolerance of this treatment was excellent for most (41 patients). Five patients experienced severe dysphagia and needed tube feeding during the treatment. One cervical abscess and 1 salivary fistula occurred for 2 other patients during radiotherapy.

TABLE 4. FUNCTIONAL ASSESSMENT OF SWALLOWING AT SIX MONTHS AND AT ONE YEAR POSTOPERATIVE

	6 Months (62 Patients Evaluable)	*1 Year (46 Patients Evaluable)*
Normal diet	38	35
Soft diet	18	10
Liquid diet	2	1
Feeding tube	4	0

Long-Term Functional Results. The results for swallowing at 6 months and 1 year postoperatively are given in Table 4. Swallowing evaluation during the following years, in evaluable patients, has shown no impairment of swallowing with time. The functional results at 1 year postoperatively in patients who had preoperative or postoperative radiotherapy are given in Table 5. There was no statistical difference between the two groups regarding the functional results. Until that point, only 2 patients needed dilations for strictures. One of these strictures occurred on a jejunogastric anastomosis in a patient who underwent esophagectomy for an esophageal second primary.

The reconstruction was achieved with only one operation in 61 cases. Seventeen patients underwent reoperation for anastomosis control, hematoma, or wound healing, and 3 for salivary fistula cure. One patient experienced a secondary exposure of the graft with salivary fistula in relation to a tracheostoma disjunction and had to undergo a reintervention 28 months later for the closure of the fistula with a deltopectoral flap. Another needed a reintervention at 24 months postoperatively to shorten a too-long bowel loop that impaired swallowing.

Regarding speech rehabilitation, the patients were mainly taught esophageal speech, and 6 prostheses were put in place through a tracheoesophageal fistula. Among the 53 evaluable patients, 7 developed

TABLE 5. FUNCTIONAL ASSESSMENT OF SWALLOWING AT ONE YEAR POSTOPERATIVE (46 PATIENTS EVALUABLE) IN RELATION TO PREVIOUS RADIOTHERAPY OR POSTOPERATIVE RADIOTHERAPY

	Previous Radiotherapy (17 pts)	*Postoperative Radiotherapy (29 pts)*
Normal diet	14 (82.4%)	21 (72.4%)
Soft diet	3 (17.6%)	7 (24.1%)
Liquid diet	0	1 (3.4%)
Feeding tube	0	0

χ^2 Test (normal diet in comparison with soft or liquid diet), not significant ($p = .44$).

TABLE 6. CARCINOLOGIC FAILURES AMONG 79 PATIENTS WHO UNDERWENT CIRCUMFERENTIAL RESECTION FOR TUMOR

	Patients	
	No.	*%*
Carcinologic failures	45	57.0
Local recurrences	7	8.9
Nodal recurrences	4	5.1
Locoregional recurrences	9	11.4
Isolated metastases	17	21.5
Second primaries	8	10.1

good, intelligible speech, 9 had fair results, and 37 had poor results.

Carcinologic Results. Ipsilateral radical or functional neck dissection was performed in 72 cases, and bilateral in 59 cases. Resected lymph nodes were recorded in 71 cases: not invaded (N–) in 26 cases (37%), invaded without perinodal extent (N+R–) in 11 cases (15.5%), and invaded with extracapsular spread (N+R+) in 34 cases (48%). The thyroid gland was partially removed (hemithyroidectomy or subtotal thyroidectomy) in 55 cases, and totally removed in 8 cases. It was found to be invaded in 12 cases. The specimen surgical margins were found to be free in 61 cases, and invaded or "questionable" (very narrow clear margins, perineural involvement, or tumor cells in the vessels) in 17 cases. The esophageal margins were involved in 5 cases, the superior margins (oropharynx) in 8 cases, and both esophageal and superior margins in 1 case. In 2 cases the margins were considered questionable in the peritracheal soft tissue, and in 1 case on the posterior pharyngeal wall.

The average length of follow-up for the 79 patients who underwent circumferential resection for carcinologic reasons is 50 months (median 38, range 4 to 160). During the period of observation, 45 patients experienced a carcinologic failure (Table 6). At the time of analysis, 25 patients are alive free of disease, 44 are dead, 3 are alive with a local or a nodal recurrence, 2 with a second primary, and 5 with metastasis. The mean time to locoregional recurrence was 10.5 months (median 11, maximum 21). The sites of local or locoregional recurrence are given in Table 7. Inferior recurrences, that is to say, local recurrences of the lower margins or tracheostomal recurrences or nodal recurrences, occurred in 7 patients. Of those patients, 2 initially had a tumor extending to the pharyngoesophageal junction (PEJ; Table 8). The mean time to metastasis was 22 months (median 13, range 4 to 151), and to second primaries, 43 months (median 24, maximum 128). The sites of metastasis (21 patients) were the lung in 15 cases, the liver in 6, the bone in 4, and other sites in 2. The sites of second primaries (8 patients) were the oropharynx in 4 cases, the lung in 2, and the esophagus in 3. The 3 esophageal second primaries occurred in patients who previously had hypopharyngeal tumors without extension to the PEJ (Table 8). The causes of death are given in Table 9. For the whole group, the 3-year survival rate was 40% and the 5-year survival rate 36%. For the patients who were N+R+, the 3-year survival rate was 28% (see Figure).

TABLE 7. SITES OF LOCAL AND LOCOREGIONAL RECURRENCES

Isolated local recurrences	
Oropharynx	4
Stomal	2
Esophagus	1
Impossible to define	1
Locoregional recurrences	
Local (oropharynx) + nodal	2
Recurrential area	4
Lymphangitic skin spread or diffuse cervical recurrence	3

TABLE 8. INFERIOR RECURRENCES (ESOPHAGEAL OR STOMAL OR RECURRENTIAL) IN PATIENTS WITH INITIALLY INVADED PEJ AND IN OTHER PATIENTS

	Tumor Originating in or Extending to PEJ (n = 34)	*Tumor Without Extension to PEJ (n = 45)*
Inferior recurrences	2 (5.9%)	5 (11.1%)
Second esophageal primary	0	3

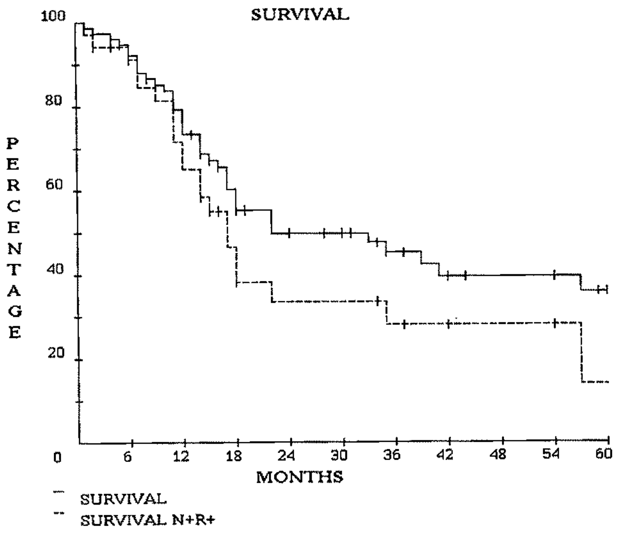
PEJ — pharyngoesophageal junction.

DISCUSSION

Today, the free transplantation of jejunum is an attractive method for single-stage reconstruction of circumferential pharyngeal defects. As a consequence of the development of microvascular techniques, this procedure has become very reliable, with a low rate of graft failure and postoperative complications.[1,5,6] Our results concerning the reconstruction success rate and the postoperative outcome are similar to those of other recent series.[2,3] They con-

TABLE 9. CAUSES OF DEATH

	Patients (N = 44)	
	No.	*%*
Local recurrences	6	13.6
Nodal recurrences	4	9.1
Locoregional recurrences	6	13.6
Metastasis	15	34.1
Second primaries	2	4.5
Postoperative	2	4.5
Intercurrent	3	6.8
Unknown	6	13.6



Survival for whole group and survival for patients with extracapsular spread (N+R+).

firm a quick swallowing rehabilitation (median time to removal of the nasogastric tube was 16 days) and a short hospital stay (median time to discharge was 23 days), which are two of the aims of this kind of reconstruction. Moreover, this procedure was convenient even in an irradiated area.[7] Previous radiotherapy (32 patients) did not increase the risk of graft failure (2/32 versus 2/53, $p = .58$). The time to swallowing rehabilitation and time to discharge were the same in the previously irradiated group.

Long-term assessment has shown in this series that FJA, like other digestive transplants, allows a good quality of swallowing: at 1 year postoperatively, no patient free of disease needed a nasogastric tube, 76% of patients were able to eat a normal diet, and 22% were able to eat a soft diet. Moreover, such good functional results do not decrease with time, and no patient experienced an impairment of swallowing function during the following years. Only 2 patients needed dilations. Other teams have reported the same experience,[8,9] pointing out the good functional results and the low rate of stenosis for this kind of reconstruction, especially when compared with skin flap reconstruction.[10,11]

Regarding speech rehabilitation, the results were poor. This was previously noticed by other teams.[3,9,11] Among the 53 patients who were taught esophageal speech, only 7 (13%) developed intelligible speech. It is probably important to avoid insetting too long a bowel loop, but even then, learning esophageal voice remains quite difficult. The number of our patients who have vocal prostheses is at present too small for us to assess this technique. Other teams reported good results with prostheses in a few cases.[3,12]

The poor radiation tolerance of the small intestine in its native location is well known. Many investigators have reported the late effects of external radiotherapy on the abdomen and pelvis, especially the risk of stricture and bowel obstruction.[13] Other animal experimental studies have demonstrated histologic changes and fibrosis due to postoperative radiotherapy in FJA.[14] Hence, the question was whether postoperative radiotherapy increased the risk of secondary stenosis in our patients. In this series, postoperative radiotherapy was mainly well tolerated. There was no statistical difference between the patients who had previous radiotherapy and those who underwent postoperative radiotherapy, regarding swallowing function, even on long-term assessment. Similar results have been reported by Cole et al.[15] Moreover, as pointed out by those authors, radiation decreases the hypersecretion of the jejunum. Therefore, full-dose postoperative radiotherapy can be relatively safely administered in patients with FJA; this is important, because radiotherapy is a major component of the treatment of advanced hypopharyngeal tumors.

Our carcinologic results confirm the poor prognosis of hypopharyngeal tumors. The main failures were locoregional recurrences (more than 25% of the patients experienced a local and/or nodal recurrence) and metastases (they occurred in 21.5% of the patients and caused more than one third of the deaths). Such results are not surprising, because hypopharyngeal tumors are known to have a high potential for locoregional spread and a high risk of metastasis.[16,17] However, the mean time to metastasis was 22 months. This is an additional argument for the use of a reconstructive method that allows one-stage reconstruction, quick swallowing rehabilitation, and a good quality of survival.

Another question to be discussed is whether it is possible to perform total pharyngolaryngectomy with cervical esophagectomy without total esophagectomy in patients with a tumor spreading to the PEJ, that is to say, the inferior border of the cricoid. In this series, we did not systematically turn down such cases for the above-mentioned resection. Indeed, we perform a circumferential pharyngolaryngectomy without total esophagectomy under two conditions: when the endoscopic assessment has eliminated a simultaneous esophageal primary and when the patient's conformation allows large inferior resection margins. Technically speaking, when the resection does not go beyond the level of the thoracic inlet, it is always possible to perform a reconstruction with the FJA. From a carcinologic point of view, the rationale for total esophagectomy in such cases is submucosal spread of the tumor when the PEJ is involved and the risk of second esophageal primaries. However, there is no reason to think that extension to the PEJ increases the risk of esophageal simultaneous primaries. In our series, the 3 esophageal second primaries occurred in patients who previously had a hypopharyngeal tumor without extension to the PEJ. Gluckman et al[18] have reported a series of 43 hypopharyngeal tumors extending to the PEJ resected with a circumferential pharyngectomy. No second esophageal primaries occurred.[18] Collin and Spiro,[19] in their series (71 patients), discovered multifocal carcinoma in 23% of the removed esophagi, but the tumors were cervical esophageal tumors and they did not indicate whether there was a tumor at the endoscopic assessment or if the tumors were only a histologic discovery. Kakegawa et al,[20] in their series of 29 tumors of the cervical esophagus, found no second primaries on the entire esophagus at histologic study. Otherwise, in the series of Ho et al,[21] the 3 esophageal second primaries occurred in patients who underwent total laryngectomy with partial pharyngectomy. None occurred in patients with circumferential pharyngolaryngectomy, even if these authors, like us, performed this resection when the tumor had spread to the mucosa of the inferior border of the cricoid.[21] Therefore, it may be thought that modern investigations allow an efficient discovery of second primaries and that extension to the PEJ does not cause a higher risk of esophageal second primaries. The other problem is the risk of submucosal spread, which could lead to increased risk of local inferior recurrence. In their series, Ho et al[21] and Gluckman et al[18] pointed out that local recurrences are mainly oropharyngeal. Davidge-Pitts and Mannel,[22] in their histologic study, found that the maximum submucosal spread in such tumors is 30 mm. In our series, as in the series of Ho et al,[21] the rate of inferior recurrences is not higher for patients who had a tumor extending to the PEJ. On the other hand, the operative risk of a total esophagectomy must be taken into account. Even if, in recent series, the mortality rate of total esophagectomy has become lower, the morbidity remains important.[17,21,23,24] Further, as pointed out by Lam et al,[25] the decrease in postoperative complications is due, in part, to more careful selection of patients. Total esophagectomy "on principle," in our opinion, does not offer sufficient carcinologic advantages to be justified in all cases of spread to the PEJ. In carefully selected cases, it is possible to perform a "limited" surgery with no impairment of carcinologic results.

CONCLUSION

This series of 83 cases confirms that the FJA is a successful method of reconstruction of the digestive tract after total pharyngolaryngectomy with cervical esophagectomy. It allows single-stage reconstruction, quick swallowing rehabilitation, and good long-term functional results. Moreover, this graft tolerates postoperative radiotherapy without impairing the swallowing function. Carcinologic results showed a high rate of locoregional recurrences and metastasis with a poor prognosis. Such results advocate for a reconstructive option that provides quick rehabilitation and an acceptable quality of survival.

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TRAUMATOLOGY OF THE HEAD AND NECK

The Berlin Course on Traumatology of the Head and Neck (with osteosynthesis exercises and live operation) will be held November 5-7, 1998, in Berlin, Germany. The special topic will be acceleration-deceleration trauma (whiplash). For information, contact Prof Arne Ernst, Dept of Otolaryngology at UKB, Rapsweg 55, D-12683 Berlin, Germany; telephone 030 5681 2801; fax 030 5681 2903; e-mail ernst.orl.ukberlin@t-online.de.

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SURGICAL TREATMENT OF LARYNGOTRACHEAL STENOSIS: A REVIEW OF 60 CASES

JAVIER GAVILÁN, MD

MARÍA A. CERDEIRA, MD ADOLFO TOLEDANO, MD

MADRID, SPAIN

Management of laryngotracheal stenosis (LTS) remains one of the most challenging problems facing the otolaryngologist. The key to success is to obtain adequate rigid circular support with normal mucosal lining. Sixty patients with LTS were surgically treated in our institution over a 20-year period. Most patients were adults; the mean age was 29 years. There were 39 males and 21 females. The most frequent cause of stenosis was intubation, in 25 patients, and tracheostomy was the cause in 21. Forty-two patients (70%) were successfully decannulated following 117 procedures in this series. An average of 1.95 procedures per patient was performed. The median time between treatment and decannulation was 561 days. These results confirm the complexity of the management of LTS. Decannulation is not always possible, and to achieve this goal, more than one treatment is frequently required. It is better to prevent LTS rather than to treat it once it has occurred.

KEY WORDS — laryngeal stenosis, laryngotracheoplasty, surgery, tracheal stenosis.

Laryngotracheal stenosis (LTS) includes a wide spectrum of lesions from the supraglottis to the lower trachea. The most common cause of LTS is trauma to the airway, either from an intubation injury or from an external source, eg, blunt trauma or tracheostomy.[1] The consequence of the injury is frequently a complex lesion including multiple sites along the upper airway (ie, glottis, subglottis, trachea), compromising an accurate diagnosis and complicating the treatment. The precise identification of the location and nature of the obstruction, and the election of the appropriate procedure for its correction are the two main challenges facing the otolaryngologist.

A complete diagnostic workup must include the exploration of the entire upper airway, from the supraglottis to the carina, defining not only the site of the obstruction but also its severity and the coexistence of associated complicating factors. Vocal cord paralysis, tracheomalacia, and tracheoesophageal fistula may complicate the management of patients with LTS and should be precisely identified.

Multiple options are available for the treatment of LTS. These include intralaryngeal steroid injections, dilatations, endoscopic removal of the obstruction, and open surgical techniques.[2-5] Among the various methods available for the surgical treatment of patients with LTS, no single procedure is appropriate for all situations. The surgeon must choose the operation best suited for every particular case. This report reviews the results of the surgical treatment of LTS in 60 patients.

MATERIALS AND METHODS

Over a 20-year period (1972 to 1991), 123 patients with LTS were admitted to our institution. The medical records of 60 patients surgically treated in the department of otorhinolaryngology are reviewed. Patients managed with conservative procedures, those in whom LTS was due to intrinsic or extrinsic malignant tumors, and patients treated in other departments are not included in the study. The average patient age was 29 years (range 7 to 68), with only 8 patients under 14 years of age. There were 39 males and 21 females treated. All patients had an airway insufficiency not compatible with a normal life. Prior to surgical treatment, all patients had either a tracheostomy or an endotracheal tube in place. The diagnostic evaluation included the identification of the site of the stenosis, the length of the obstruction, and the diameter of the remaining lumen in millimeters. All parameters were assessed by means of endoscopic exploration, plain x-rays, xeroradiography, and computed tomography when available. Surgical treatment included a wide variety of procedures ranging from resection and reanastomosis to different types of laryngotracheoplasty. A number of grafts (cartilage, bone, skin) and intraluminal stents (T-tube, silicone, foam finger cot) were used. All patients had a complete follow-up, defined as the time elapsed between first operation and decannulation, treatment withdrawal, or death. The treatment period was mea-

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From the Department of Otorhinolaryngology, La Paz Hospital, Autonomous University, Madrid, Spain.

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CORRESPONDENCE — Javier Gavilán, MD, Servicio ORL, Hospital La Paz, Paseo de la Castellana 261, 28046 Madrid, Spain.

TABLE 1. CAUSES OF STENOSIS

Cause	*No.*	*%*
Endotracheal intubation	25	41.7
Tracheostomy	21	35.0
External trauma	9	15.0
Idiopathic	4	6.7
Cicatricial	1	1.6
Total	60	100

TABLE 3. SURGICAL PROCEDURES

Procedure	*No.*		
Resection and end-to-end anastomosis			5
Laryngotracheoplasty			91
Cartilage graft	80		
Hyoid bone	11		
Stents		86	
T-tubes	63		
Other	23		
Skin graft		27	
Laryngoplasty			11
Cordectomy	2		
Arytenoidectomy	3		
Cordectomy and arytenoidectomy	3		
Partial supraglottic resection	3		
Minor procedures			10
Total procedures			117
Average procedures per patient			1.95 (range 1-10)

sured in days from the first surgical procedure. Decannulation was used as a criterion for treatment success. Voice quality was also assessed at first evaluation and at the end of the treatment, except for patients with endotracheal intubation at the time of the first visit. However, this parameter was evaluated on a subjective basis and was excluded from the study. Statistical analysis was performed by the χ^2 test and Fisher's exact test with the StatView v4.01 program. All statistical tests received the same level of significance of .05.

RESULTS

The cause of stenosis was prolonged or traumatic endotracheal intubation in 25 patients, tracheostomy in 21, trauma in 9, cicatricial in 1, and idiopathic in 4 (Table 1). The sites of stenosis are listed in Table 2. Single stenosis, either subglottic (10) or tracheal (16), occurred in 43.3% of the patients. Combined laryngotracheal obstruction was present in 33 patients. One patient had an isolated supraglottic stenosis. The average length of the lesion was 17 mm, with a range of 4 to 50 mm. Twenty-nine patients had a complete obstruction of the airway, defined as a remaining lumen of 1 mm or less in diameter. The median diameter of the stricture was 3 mm, ranging from 0 to 10 mm. In 1 patient, a tracheoesophageal fistula complicated the stenosis and was successfully managed by a single-stage laryngotracheoplasty combined with esophageal suture and pedicled sternocleidomastoid muscle flap interposition.

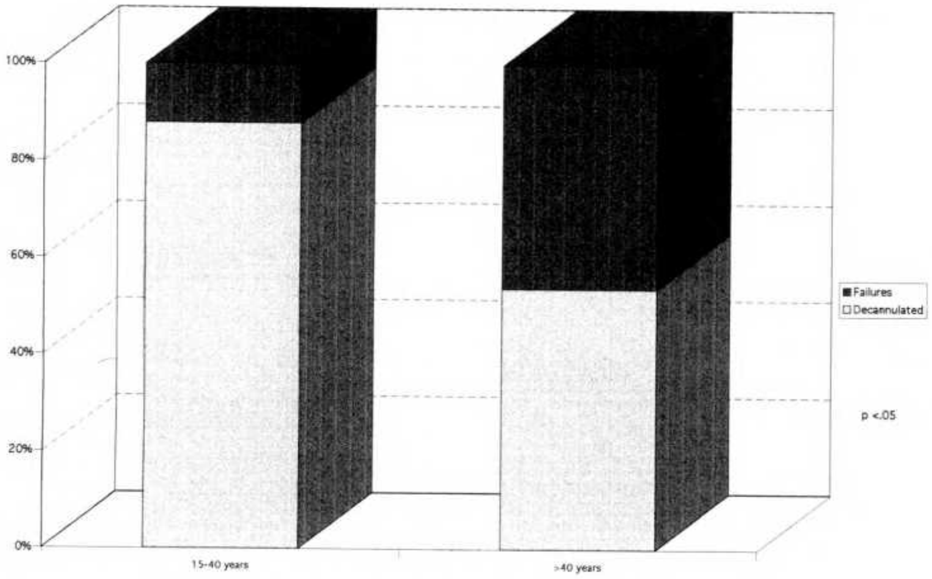
The surgical procedures performed in this series appear in Table 3. Overall, 117 operations were performed (range 1 to 10 procedures per patient), with an average of 1.95 procedures per patient. Resection of the stenotic segment and end-to-end anastomosis was performed 5 times. Different types of laryngotracheoplasty, including anterior and posterior cricoid split, were used on 91 occasions. To provide rigid support to the reconstruction, different materials were used for grafting. These included cartilage in 80 cases and hyoid bone in 11 operations. Intraluminal stents in laryngotracheoplasty were used in 86 operations, including 63 T-tubes. Other materials, such as a foam finger cot and silicone molds, were used in 23 cases. Most T-tubes were maintained in place for 1 to 2 years. The only particular complication of such long-term stenting was the development of granulation tissue at the upper end of the tube in some patients. This was especially frequent in long T-tubes passing through the vocal cords and contacting the petiole of the epiglottis. This prob-

TABLE 2. SITES OF STENOSIS

Site	*No.*		*%*
Isolated		27	45.0
Subglottic	10		16.7
Tracheal	16		26.7
Supraglottic	1		1.6
Combined		33	55.0
Glottic-subglottic-tracheal	7		11.7
Glottic-subglottic	9		15.0
Subglottic-tracheal	13		21.7
Supraglottic-glottic	3		5.0
Supraglottic-subglottic-tracheal	1		1.6

TABLE 4. RESULTS OF TREATMENT

Result	*No.*	
Decannulated		42
Trauma	8	
Intubation	16	
Tracheostomy	14	
Other	4	
Failures		12
Trauma	1	
Intubation	7	
Tracheostomy	3	
Other	1	
Death		4
Still being treated		2



Age is only parameter with significant impact on success rate. Difference between decannulation rate of young patients and subjects over 40 years of age is statistically significant (p < .05).

lem was easily solved by removing the mold and inserting a new, shorter T-tube. Skin grafts surrounding the stent to resurface the surgical defect were used on 27 occasions. Simple laryngoplasty (without tracheal reconstruction) was required in 11 patients, including 2 cordectomies, 3 arytenoidectomies, 3 combined cordectomies plus arytenoidectomies, and 3 partial supraglottic resections. Minor procedures to correct previous operations, such as replacement of long T-tubes, were required on 10 occasions.

Table 4 shows the results of treatment. Four patients died during the follow-up before decannulation was achieved. Three of them died of unrelated causes, and 1 from a T-tube complication. Among the remaining 56 patients, 42 (75%) obtained a satisfactory airway and were decannulated. The median time between operation and decannulation in this group was 561 days, with a range of 9 days to more than 12 years. Twelve patients (21.4%) were not decannulated and continue to require a tracheostomy tube. The 2 remaining patients are still being treated. According to the cause of the stenosis, 3 patients were not decannulated in the tracheostomy group (17.7%) and 7 patients failed decannulation in the intubation group (30.4%). The 2 remaining failures belong to the trauma and idiopathic groups.

TABLE 5. DECANNULATION RATE

Parameter	%	*p*
Age		<.05
≤40 years	87.9	
>40 years	53.8	
Cause of stenosis		NS
Intubation	69.6	
Tracheostomy	82.3	
External trauma	88.9	
Length of stenosis		NS
<20 mm	83.3	
20-35 mm	75.0	
>35 mm	50.0	
Type of stenosis		NS
Isolated	85.2	
Combined	70.4	

NS — not significant.

Age is the only parameter with a significant effect on the success rate among patients who completed the treatment. Excluding the pediatric population, two similar age groups were created (range 15 to 68). Subjects over 40 years of age had a decannulation rate of 53.8% (7/13), while patients between 15 and 40 years of age were decannulated in 87.9% (29/33) of the cases (p < .05; see Figure). Sex, cause of the stenosis, preoperative diameter of the stricture, length of the stenotic segment, and site of stenosis — isolated versus combined — had no significant effect on the outcome of the procedure (Table 5). Despite a lack of statistical significance, some figures deserve special attention. Concerning the cause of LTS, a large number of failures (7/12) appeared in the group of stenoses produced by prolonged intubation. The decannulation rate was 69.6% (16/23) in the intubation group, 82.3% (14/17) in the tracheostomy group, and 88.9% (8/9) in the group of patients with LTS of traumatic origin. The length of the stenotic segment showed an inverse relationship to the success rate. Patients with stenoses shorter than 20 mm were decannulated in 83.3% of cases (25/30). The success rate decreased to 75% in patients whose stenoses ranged between 20 and 35 mm in length (15/20), and only 50% of the patients with stenoses longer than 35 mm achieved decannulation

(2/4). Concerning the type of stenosis, 8 of 27 patients with combined stenoses failed decannulation (29.6%), while only 4 of 27 patients with isolated stenoses were treatment failures (14.8%).

DISCUSSION

Improvements in respiratory care and better knowledge of biomechanics following tracheostomy have resulted in a substantial decrease in the reported incidence of LTS.[6] However, laryngotracheal injuries remain a feared complication of prolonged intubation and tracheostomy. External trauma to the upper airway is an increasing cause of LTS. Treatment options for LTS may be classified into conservative procedures and open surgical techniques. Conservative procedures may be useful in simple, uncomplicated LTS. However, severe circumferential stenoses exceeding 1 cm in length with destruction of the cartilaginous support of the upper airway should be managed with open procedures. Patients with an important glottic or subglottic compromise are also candidates for an open technique. The key to success with open surgical techniques remains the creation of an adequate rigid circular support with normal mucosal lining.

The results described in this paper are similar to others reported in the literature[5,7] and could be considered satisfactory in terms of decannulation. Three of 4 patients who completed treatment obtained a satisfactory airway and were decannulated. Unlike in other reports, age was the only significant factor regarding success in this study. To avoid the mixing of pediatric cases with adults, all patients under 15 years of age were excluded from this comparison. Although the length of the stenosis did not have a significant impact on decannulation, it showed an inverse relationship with the success rate. The lack of statistical significance of this parameter could be due to the small number of patients with long stenotic segments included in the study.

However, we are not satisfied with some details in this series, namely, the number of operations per patient and the time between first treatment and decannulation. Subglottic and combined stenoses constitute a complex problem because of the special structural characteristics of the cricoid and its important anatomic relationships. Two options are available for stenosis with important subglottic involvement: laryngotracheoplasty and thyrotracheal anastomosis.

Laryngotracheoplasty is a general term describing different operations used to repair LTS. In 1956, Rethi[8] introduced the concept of surgical expansion of the cricoid cartilage and stressed the importance of not disturbing scar tissue within the larynx, establishing the basis for modern-day laryngotracheoplasty. The Rethi procedure underwent numerous modifications that include the use of different grafts for airway augmentation, multiple stents, and a variety of prostheses.[9] The large number of different types of laryngotracheoplasty is evidence of the great difficulties involved in the management of these patients.

Laryngotracheoplasty was the standard treatment for patients with subglottic and combined stenoses in this series. Despite its acceptable results concerning decannulation, the duration of treatment — with a median time between first treatment and decannulation of more than 18 months — seems excessive. This could be mainly attributed to a very prolonged stenting time. In most patients undergoing laryngotracheoplasty, a stent was used as a lumen keeper. In the case of T-tubes — 73% of the stents — the mold was maintained in place for 1 to 2 years. Although no particular significant complications other than granulation tissue at the upper end of some T-tubes could be related to such long-term stenting, we currently think there is no need for such prolonged stenting in laryngotracheoplasty. The main goals of intraluminal stenting are obtained within the first 4 to 12 weeks after the operation.[9] Structural support is provided by cricoid grafting, with the stent acting during the first weeks to help stabilize the reconstructed airway. When skin grafts are used, stenting is also necessary for approximation and immobilization of the graft to the exposed vascularized tissue in order to facilitate graft adherence and survival.[10] This goal is obtained after 7 days of stenting. Then, bacterial growth develops rapidly, resulting in progressive skin graft necrosis.[11] Also, better voice results have been reported when the duration of stenting was 12 weeks or less.[9]

The second unsatisfactory result of this review is the overall number of operations performed in the patients of this series, with almost 2 operations per patient. This is a well-known disadvantage of laryngotracheoplasty, with an even higher number of surgeries per patient reported in the literature.[7] The majority of our failures (10/12) occurred in patients with subglottic involvement, thus reflecting the complexity of this particular type of LTS. In the mid-1970s, Gerwat and Bryce[12] and Pearson et al[13] described and popularized the segmental resection and primary thyrotracheal anastomosis for the surgical management of subglottic stenosis. Although initially considered dangerous, excellent results have been reported with this technique, especially in patients with normal vocal cord mobility in whom a posterior shell of cricoid plate could be preserved.[14,15] Thyrotracheal anastomosis was not performed in patients in this series. However, in light of the results reported in this paper, we have experienced a trend toward a more aggressive approach in patients with extensive subglottic involvement, in an effort to reduce the treatment time and number of operations while maintaining or increasing the decannulation rate.

Cervical and lower tracheal stenoses are usually easier to solve than subglottic stenosis. In patients with isolated tracheal stenosis, the best way to obtain a stable ring structure to support the airway is by segmental resection and end-to-end anastomosis. This approach was seldom used in this series, with only 5 procedures in 16 patients with isolated tracheal stenosis. The reason for this was the widespread use of T-tubes, which were considered a standard treatment of LTS during several years in our department. However, the good results of tracheal resection and end-to-end anastomosis in 5 patients successfully decannulated, 3 of them after multiple unsuccessful laryngotracheoplasties, led us to a modification in the approach to tracheal stenosis. We currently believe that segmental resection and end-to-end anastomosis is the best option for most cases of isolated tracheal stenosis. Stenotic segments of up to 3 cm can be resected with this method, without releasing maneuvers, in young patients with elastic annular ligaments. When the gap resulting after resection of the stenosis cannot be closed without tension, releasing techniques must be used in order to obtain a tension-free approximation of both tracheal stumps.[16]

CONCLUSION

The management of LTS remains a major problem for the head and neck surgeon. Several situations such as vocal cord paralysis, tracheomalacia, and tracheoesophageal fistula may complicate the management of patients with LTS and should be precisely identified and included in the treatment plan. The surgeon needs flexibility to modify intraoperatively any preoperative plan, and experience to adjust the operation to the individual situation. Despite the large number of different open surgical procedures available for the treatment of severe LTS, no single procedure is appropriate for all situations, and the surgeon must choose the operation best suited for every particular case. On the basis of our 20-year experience in the management of severe LTS, we now recommend segmental resection and end-to-end anastomosis in order to decrease the treatment time and reduce the number of procedures per patient. This approach, combined with a rational use of laryngotracheoplasty and intraluminal stenting for complex combined lesions, should improve the results of the management of severe LTS.

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PARTIAL VERTICAL LARYNGECTOMY IN THE TREATMENT OF EARLY GLOTTIC CANCER

TZUNG-SHIAHN SHEEN, MD

JENQ-YUH KO, MD

YEN-LIANG CHANG, MD

TAIPEI, TAIWAN

Treatment of early glottic cancer is still controversial. A retrospective study was conducted to analyze the results of 49 partial vertical laryngectomies in our department. All patients except 1 were successfully decannulated. The quality of voice was suboptimal but serviceable in all patients. Overall, there were 9 recurrences: 7 in the larynx and 2 in the neck. In the laryngeal recurrence group, 5 of 6 patients were successfully salvaged with total laryngectomy. One patient died of acute renal failure 1 month after salvage operation. Another patient survived 4.5 years after cisplatin-based chemotherapy. In the neck recurrence group, 1 patient was successfully salvaged with neck dissection; the other was lost to follow-up after neck dissection. The actuarial 5-year survival rate was 97.4% and the larynx preservation rate was 88%. The recurrence rate was 14%, when 2 of the 7 laryngeal recurrences were excluded because they occurred beyond 5 years. The results of this series were encouraging. We suggest that partial vertical laryngectomy is a good treatment modality for selected early glottic cancers, especially those of the T1 group.

KEY WORDS — glottic cancer, laryngeal cancer, partial vertical laryngectomy.

INTRODUCTION

Early glottic cancer is defined as a carcinoma that originates from the glottis without fixation of the vocal cords. T1 and T2 glottic cancers are categorized in this group. Radiotherapy or conservation laryngeal surgery is the mainstay of treatment. There have been many discussions on the management and results with respect to different treatment modalities. However, controversy still exists, and treatment varies from institution to institution.[1-5] In our hospital, partial vertical laryngectomy (PVL) and endoscopic stripping with or without laser vaporization are the major surgical procedures for early glottic cancers. Major indications for PVL are large invasive T1 glottic tumors, especially those that invaded the anterior commissure, some small T2 tumors with minimal supraglottic or subglottic extension, tumors without involvement of more than one third of the opposite vocal cord, and tumors that are difficult to visualize by endoscopy. The tumor should not involve the body of the arytenoid or extend more than 1 cm below the vocal cord. We have used PVL since 1976, and the results have been very encouraging.

MATERIALS AND METHODS

A retrospective study was conducted to evaluate the efficacy of PVL for early glottic cancer in our department. The indications for PVL were similar to those of other series.[6] In addition, recurrent glottic cancer after failure of radiotherapy was also included.

There were 49 patients with early glottic cancer who underwent this operation in the period from 1980 to 1995. All patients except 1 were previously untreated. The age of these patients ranged from 36 to 75 years, with an average age of 59.5 (median 61.2). All the tumors were diagnosed clinically as T1 (n = 38) or T2 (n = 11) glottic tumors. All patients passed preoperative spirometry screening. The period of follow-up ranged from 3 months to 15 years, with a median of 6.0 years.

The operative procedures were similar to those of other reports,[6-8] with some modifications. All patients underwent tracheotomy under local anesthesia. A transverse skin incision along the cervical skin crease was performed at the midthyroid level from the anterior border of one sternocleidomastoid muscle to the other. After elevation of the cervical skin flaps, the cervical fascia was split and the strap muscles were separated from the thyroid cartilage to expose the laryngeal cartilaginous framework. The perichondrium of the thyroid cartilage was elevated after midline, superior, and inferior incisions were made. A median or paramedian vertical cartilaginous cut, depending on the clinical situation, was performed on the thyroid cartilage. The larynx was exposed via the cricothyroid membrane and then it was opened like a book. The posterior cartilaginous cut was made according to the extent of the tumor, usually anterior to the insertion of the inferior pharyn-

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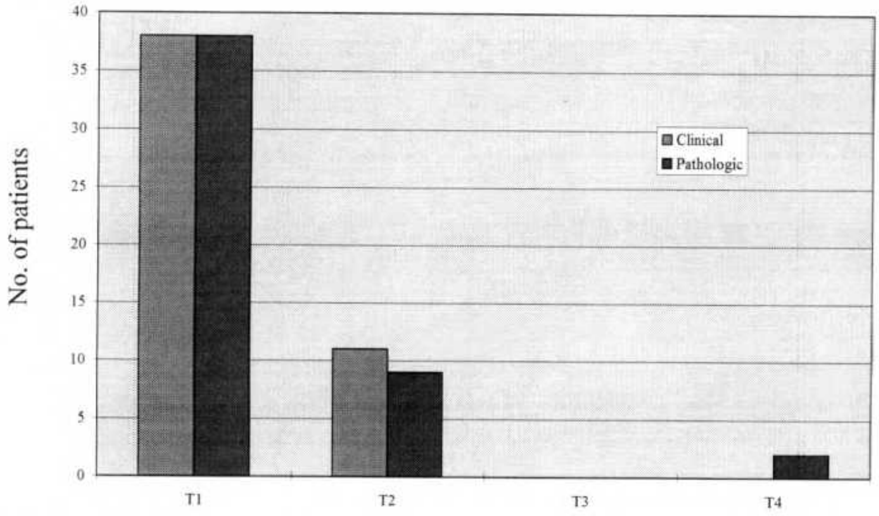
From the Department of Otolaryngology, National Taiwan University Hospital, Taipei, Taiwan.

CORRESPONDENCE — Tzung-Shiahn Sheen, MD, Dept of Otolaryngology, National Taiwan University Hospital, No. 7, Chung-Shan South Road, Taipei, Taiwan.



Clinical and pathologic classification of 49 early glottic cancers.

geal constrictor muscle. Finally, the resection was made under direct vision. The arytenoid was removed, depending on the extent of the tumor. In most patients, the arytenoids were preserved. Intraoperative frozen section for adequate margin control was undertaken whenever the resection margin was close to the tumor. Most patients underwent laryngeal reconstruction by use of an epiglottis flap[9] or an inferiorly based sternohyoid muscle flap.[8] We did not use a laryngeal keel for prevention of adhesion or stenosis. A Penrose drain was inserted for drainage at the end of the operation.

The patients were fed with a nasogastric tube for about 2 weeks in the postoperative period. The tracheotomy tube was usually decuffed on postoperative day 5 and removed on day 7. If the patient still suffered from aspiration, the tubes were kept for a longer period until the problem resolved. Swallowing and phonation therapy usually started about 2 weeks after operation.

RESULTS

Of the 49 patients, 28 underwent reconstruction by use of an epiglottis flap, 13 had a sternohyoid muscle flap, and 8 had no reconstruction. All patients were successfully decannulated except 1, who suffered from dyspnea 2 years later that required a tracheotomy. Although the quality of voice was suboptimal in most patients, all patients could communicate without much difficulty. All the patients could tolerate oral solid and liquid intake.

The operative complication rate was minimal. One patient developed postoperative upper gastrointestinal tract bleeding and was successfully treated with conservative therapy. A few patients developed a wound infection, and all were treated successfully with conservative methods. A few patients required postoperative endoscopy to remove laryngeal granulation tissue. There was no difference in terms of complications or quality of life between groups of patients with different methods of laryngeal reconstruction.

Of the 49 tumors, 38 were diagnosed as clinical T1 tumors, and 11 as T2 tumors. Two of the clinical T2 tumors were demonstrated to be pathologic T4 tumors. The case numbers of final pathologic classification were 38, 9, and 2 for T1, T2, and T4, respectively (see Figure).

There were 9 recurrences: 7 in the larynx and 2 in the neck (Table 1). None of them underwent intraoperative frozen section examination. The overall recurrence rate was 18% (10.5% and 45.5% for clinical T1 and clinical T2 tumors, respectively). However, the true recurrence rate was 14% (5.2% and 45.5% for clinical T1 and clinical T2 tumors, respectively) when 2 of the laryngeal recurrences that occurred beyond 5 years were excluded because they were probably second primaries.

In the laryngeal recurrence group, 5 of 7 patients were successfully salvaged with total laryngectomy. One patient (patient 4) died of acute renal failure 1 month after a salvage operation. One (patient 5) was treated with chemotherapy. This patient had tumor recurrence and a suspected lung metastasis 8.3 years after primary operation. He underwent monthly cisplatin and 5-fluorouracil combination chemotherapy for 6 months. He survived 4.5 years after chemotherapy. In the neck recurrence group, both patients were salvaged with neck dissection. One of them moved to another hospital for postoperative radiotherapy and was lost to follow-up.

In all, there were 5 patients (4 clinical T1 tumors and 1 clinical T2 tumor) who underwent intraoperative frozen section for adequate margin control. None

TABLE 1. RECURRENCE AFTER PARTIAL VERTICAL LARYNGECTOMY

Patient	*Age (y)*	*Sex*	*Clinical T Classification*	*Pathologic T Classification*	*Recurrence*	*Interval After Primary Treatment (y)*	*Management of Recurrence*	*Results*	*Comments*
1	46.7	M	I	I	Larynx, contralateral side	9.1	TL	L&W	May consider as second primary cancer
2	56.7	M	I	I	Larynx	0.8	TL	L&W	Positive margin on primary operation
3	59.4	M	II	II	Larynx	1.1	TL	L&W	
4	61.2	M	II	II	Larynx	2.7	TL, MRND	DOD	Died of acute renal failure after salvage operation
5	54.9	M	I	I	Larynx	8.3	Chemotherapy	DOD	May consider as second primary cancer; died 4.5 years after recurrence
6	63.3	M	I	I	Larynx	1.4	TL	L&W	
7	45.2	M	II	II	Larynx	0.3	TL	L&W	Positive margin on primary operation
8	56.9	M	II	IV	Neck, same side	2.9	RND, radiotherapy	L&W	
9	61.7	M	II	II	Neck, same side	6.7	MRND	Lost to follow-up	Patient underwent radiotherapy at another hospital after salvage operation

TL — total laryngectomy, L&W — live and well, MRND — modified radical neck dissection, DOD — died of disease, RND — radical neck dissection.

of them had recurrences.

There were 6 patients who demonstrated positive margins on the permanent pathologic studies (Table 2). One patient was lost to follow-up after completion laryngectomy; 2 patients underwent postoperative radiotherapy, and they were free of disease for 1 and 7.8 years, respectively. Three patients did not undergo any adjuvant therapy, and of these, 2 patients had recurrences at 4 and 10 months, respectively. The last patient was still free of tumor after 5 months' follow-up.

Two patients (Table 1, patients 4 and 5) died of disease in this series, 1 of whom survived beyond 5 years. The actuarial 5-year survival rate was 97.4% and the larynx preservation rate was 88% (43/49).

DISCUSSION

What is the preferred treatment for an early glottic cancer? This has been controversial; nevertheless, surgical treatment is popular in some institutions. One advantage is that we can obtain a specimen for better histologic analysis. For example, there were 2 tumors in this series that were diagnosed T2 clinically but T4 pathologically. Without pathology, we might understage these tumors, especially those in the anterior commissure. We can also save irradiation for further treatment, because the incidence of second primary cancer is quite high in this group of patients. The only disadvantage is the quality of

TABLE 2. OUTCOMES OF PATIENTS WITH POSITIVE MARGINS ON PERMANENT PATHOLOGIC STUDIES

Patient	*Age (y)*	*Sex*	*Clinical T Classification*	*Pathologic T Classification*	*Subsequent Management*	*Outcomes*
A	49.7	M	II	IV	Completion laryngectomy	Lost to follow-up after completion laryngectomy
B	58.1	M	I	I	Radiotherapy 60 Gy	Free of disease for 7.8 years
C	55.9	M	I	I	Radiotherapy 60 Gy	Free of disease for 1 year
D	56.7	M	I	I	None	Recurred 10 months later; successfully salvaged with total laryngectomy for 7.6 years
E	45.2	M	II	II	None	Recurred 4 months later; successfully salvaged with total laryngectomy for 3 months
F	58.7	M	I	I	None	Free of disease after follow-up for 5 months

voice. Although the voice quality is better in the radiotherapy group, the ultimate larynx preservation rate is less than in the surgical group.[4] Besides, the postradiotherapy follow-up is much more difficult, since it is hard to differentiate postirradiation edema from recurrence by indirect or fiberoptic laryngoscopy.

In our institution, for early glottic cancer, we let patients choose the means of treatment after thorough discussion. About one third of patients choose surgical treatment. We have used PVL since 1976. In our first series,[10] from 1976 to 1983, there were 34 patients who underwent PVL. The actuarial 3-year survival rates were 94.4% and 93.8% for T1 and T2 glottic cancers, respectively. In the T1 group, the results were similar to those of the patients who underwent radiotherapy (94.4% versus 90.9%) in the same period. In the T2 group, the results were better with surgery (93.8% versus 73.9%). In the present series, the actuarial 5-year survival rate was 97.4% and the larynx preservation rate was 88% (43/49). These results are better than those of our previous series (but without statistical significance).

There were 9 recurrences in this series: 7 in the larynx and 2 in the neck (Table 1). The overall recurrence rate was 18% (10.5% and 45.5% for clinical T1 and clinical T2 tumors, respectively). Two of the 7 laryngeal recurrences occurred beyond 5 years. The recurrence rate was 14% when these 2 patients are excluded because they were probably second primaries. One of the 2 clinically T2 but pathologically T4 tumors recurred in the neck. The tumor was located at the anterior commissure and penetrated through the thyroid cartilage without fixation of the vocal cords. The patient did not undergo postoperative radiotherapy. Postoperative radiotherapy might be necessary for those patients with clinical T2 but pathologic T4 tumors. For those patients whose pathology did not show invasion of the thyroid cartilage or the cervical soft tissues, prophylactic postoperative radiotherapy was not necessary.[11]

A negative histologic margin did not guarantee prevention of recurrence, because all except 2 of the recurrences had negative margins on the permanent-section pathologic report. Close postoperative follow-up is the best policy for early detection of recurrence. Whenever voice change or dyspnea has occurred on the follow-up visits, laryngofiberoscopy or direct laryngoscopy should be undertaken for detailed examination. Of the 5 patients who underwent intraoperative frozen section examination, none had recurrence. We strongly recommend intraoperative frozen section for adequate resection margin control.

There were 6 patients whose pathology revealed a positive margin (Table 2). One patient underwent completion laryngectomy[12] 1 week after primary operation. He was lost to follow-up thereafter. Two patients underwent postoperative radiotherapy. These 2 patients did not have recurrences in the follow-up period. Three patients were carefully followed up as recommended by Bauer et al[13]: 2 patients had recurrences 4 and 10 months later, respectively, and the third patient was still free of tumor after 5 months' follow-up. Completion laryngectomy or postoperative radiotherapy should be delivered in this group of patients because of the high recurrence rate.

A previously irradiated patient was successfully salvaged with PVL. This patient underwent radiotherapy 1 year earlier, when a T1 glottic carcinoma was diagnosed. The patient failed after 6-Gy radiotherapy was administered to the larynx. He was successfully salvaged and was free of disease 2 years after the operation. Conservation laryngeal surgery is still feasible for early glottic cancers that fail radiotherapy, as mentioned by other authors.[14,15]

When the costs of the treatments are compared, in Taiwan, they are $5,000 and $3,000 (US dollars) for radiotherapy and PVL, respectively. Besides, it takes 2 months to complete radiotherapy, while it takes only 2 to 3 weeks for PVL. When the preoperative and postoperative quality of voice was compared, all patients were satisfied. However, we cannot get preoperative and postoperative sonospectrography in most patients for objective comparison.

In this series, the actuarial 5-year survival rate is 97.4% and the larynx preservation rate is 88%. This is comparable with the findings in other groups.[5] On the basis of these results, although this is not a randomized clinical study, we suggest that PVL is a good treatment for some selected early glottic cancers, especially those of the T1 group.

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SKULL METASTASIS FROM RENAL CELL CARCINOMA

CASE REPORT AND LITERATURE REVIEW

HRAIR A. KOUTNOUYAN, MD
OAKLAND, CALIFORNIA

GREGORY J. RUMORE, MD
OAKLAND, CALIFORNIA

JONATHAN M. KAHN, MD
MARTINEZ, CALIFORNIA

Metastatic tumors to the head and neck are uncommon. We report a case of renal cell carcinoma that presented as an epidermal inclusion cyst on the forehead. The forehead is an exceedingly uncommon site for renal cell metastases. We review the biology of tumor metastases and explore the pathways by which infraclavicular tumors spread to the head and neck. We then discuss the clinical appearance and management of metastatic renal cell carcinoma.

KEY WORDS — carcinoma, head and neck neoplasms, metastasis, neoplasm, renal cell.

CASE REPORT

A healthy, 33-year-old woman was referred to the head and neck surgery department for evaluation of a mass on the left side of the forehead that was clinically consistent with a benign cyst. The mass had been present for 4 months, and the patient had no associated pain, headache, visual changes, or history of sinus disease or forehead trauma. She had no ongoing medical illnesses or physical complaints; specifically, she did not have dysuria, hematuria, or pain in the flank or abdomen. The surgical history included cholecystectomy, cesarean section, and hysterectomy for benign disease.

At examination in the head and neck surgery department, the forehead mass measured about 6 cm, was nontender and nonfixed, and had a cystic character (Fig 1). The patient's visual fields were full, and results of neurologic examination were normal. An attempt at excision was abandoned when extensive bleeding and skull erosion were encountered. Biopsy specimens were obtained, and a computed tomography (CT) scan showed a contrast-enhancing cystic lesion of the left frontal skull with marked bone destruction, intracranial tumor extension with compression of dura, and invasion of the soft tissues of the scalp (Fig 2). Using light microscopy, the pathologist interpreted the specimen as an aggressive

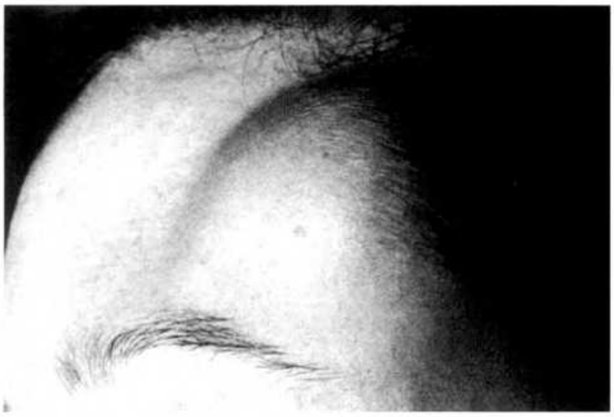


Fig 1. Photograph shows 6-cm soft tissue mass on left side of forehead.

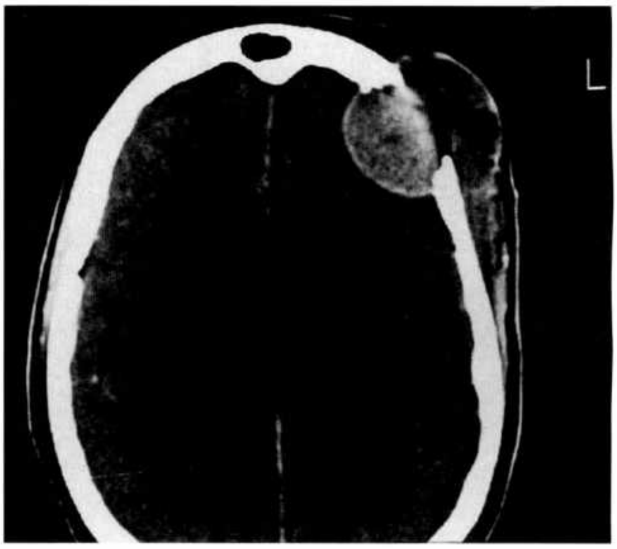


Fig 2. Computed axial tomographic view with intravenous contrast agent shows contrast-enhancing cystic lesion of left frontal area of skull with marked bone destruction, intracranial extension with compression of dura, and invasion of soft tissues of scalp.

From the Departments of Head and Neck Surgery (Koutnouyan) and Pathology (Rumore), Kaiser Permanente Medical Center, Oakland, and the Department of Head and Neck Surgery, Kaiser Permanente Medical Center, Martinez (Kahn), California.

CORRESPONDENCE — Jonathan M. Kahn, MD, Dept of Head and Neck Surgery, Kaiser Permanente Medical Center, 200 Muir Rd, Martinez, CA 94553-4696.

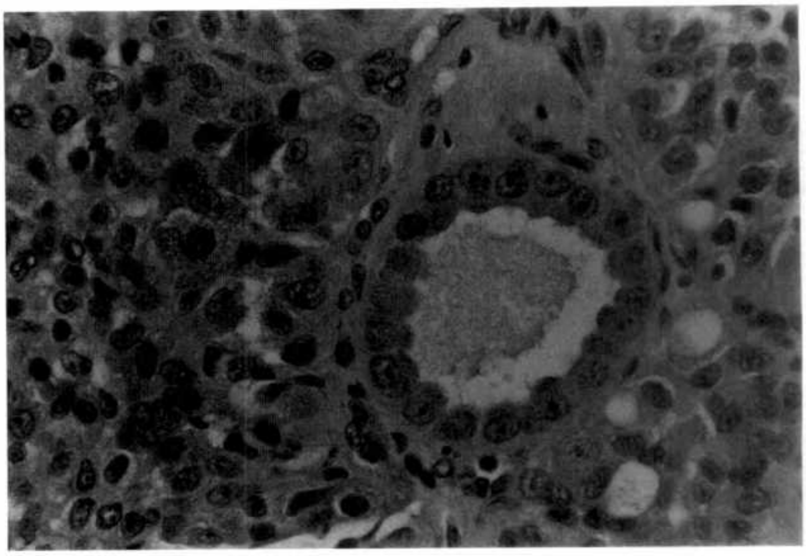


Fig 3. Histologic slide of neurosurgical specimen shows large cells with abundant granular eosinophilic cytoplasm and marked nuclear atypia with prominent nucleoli (H & E, original ×400). Cells are arranged in predominantly tubular pattern.

meningioma versus well-differentiated carcinoma.

Results of a chest x-ray examination, complete blood count, serum electrolyte levels, and results of urinalysis were normal. Neurosurgical consultation was obtained, and the patient had a craniotomy and gross debulking of the tumor. The neurosurgeon described a lesion that bled easily, affected the temporalis muscle, eroded the skull, and invaded the dura. Frozen sections showed carcinoma indicative of a metastatic process; therefore, no attempt was made to find clear margins or to resect dura. Permanent sections showed large cells with abundant granular eosinophilic cytoplasm and marked nuclear atypia with prominent nucleoli. Because these cells were arranged in a predominantly tubular pattern, the diagnosis of adenocarcinoma was considered (Fig 3). Positive immunohistochemical staining for cytokeratin confirmed that diagnosis.

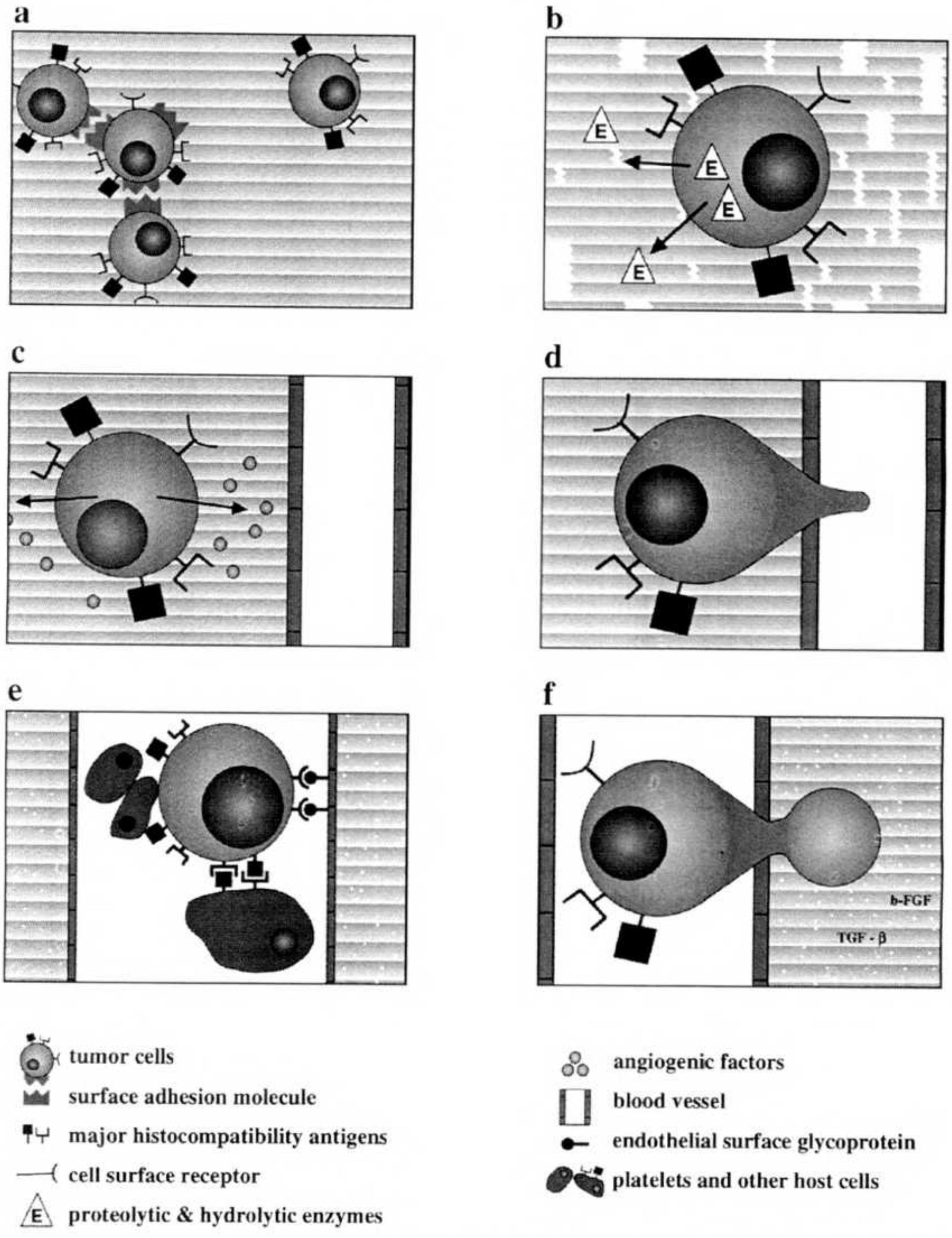
Pelvic, abdominal, and chest CT scans showed a lower-pole mass in the left kidney, bulky para-aortic and pelvic adenopathy, mediastinal adenopathy, and lytic lesions affecting the sacroiliac joints bilaterally. A CT-guided biopsy of the renal mass showed a high-grade renal cell carcinoma with granular and collecting duct features. A bone scan confirmed increased radionuclide activity in the skull and sacroiliac joints consistent with metastatic bone disease.

The diagnosis of metastatic renal cell cancer was established, and the patient sequentially received subcutaneous treatment with interferon-α (1 to 5 million IU/m^2 3 times weekly), intravenous treatment with vinblastine sulfate (6 mg/m^2 weekly), and oral treatment with megestrol acetate (800 mg daily). The disease did not respond, however, and new lytic lesions of the skull, erosion of the orbital roof, and further intracranial extension of the tumor developed. New neck adenopathy developed, and the mediastinal and pelvic adenopathy progressed. The patient sustained pathologic fractures of the right clavicle, the left humerus, and the right femur; the last two fractures required open reduction and internal fixation, and all three fractures received palliative irradiation. Treatment with a bone resorption inhibitor, pamidronate disodium (90 mg monthly), partially alleviated the pain caused by the osteolytic lesions. Nine months after the diagnosis of metastatic renal cell carcinoma was established, the patient died of systemic disease despite courses of immunotherapy, chemotherapy, and hormonal therapy.

DISCUSSION

Cancer metastasis is a selective and regulated process. Only a subpopulation of cells in a primary tumor have the genetic, biochemical, immunologic, and biologic properties to metastasize, and they do so discriminately to selective distant sites.[1,2] These cells break away from the primary tumor mass by deleting certain adhesion molecules from their surfaces (Fig 4a,b). They then degrade the host extracellular matrix with various proteolytic and hydrolytic enzymes and migrate in the direction of the degraded matrix by using chemotactic signals of the host, as well as of the tumor cells. In the process, these cells stimulate angiogenesis, which further enhances tumor growth because of growth factors produced by the endothelium of the new blood vessels. In addition, these blood vessels are more permeable to tumor cells than are native blood vessels and therefore facilitate entry of tumor cells into the circulation (Fig 4c,d).

After entering the bloodstream, most tumor cells are destroyed by the host surveillance apparatus. A few tumor cells survive by expressing host histocompatibility antigens and by evading the circulating cy-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b-FGF
TGF - β
tumor cells
surface adhesion molecule
major histocompatibility antigens
cell surface receptor
E proteolytic & hydrolytic enzymes
angiogenic factors
blood vessel
endothelial surface glycoprotein
platelets and other host cells

Fig 4. Schematic depicts cellular events during metastasis. a-d) Background shows extracellular matrix at primary tumor site. a) Cell breaks away from primary tumor after losing surface adhesion molecule. b) Hydrolytic and proteolytic enzymes degrade native extracellular matrix. c) Cell stimulates angiogenesis. d) Cell extravasates into circulation. e,f) Background shows extracellular matrix at metastatic site. e) Cell arrives at target capillaries and is trapped by endothelium. Other cells aggregate, and thrombus forms. f) Cell extravasates through basement membrane into target extracellular matrix. Local growth factors enhance proliferation.

totoxic T cells. Other tumor cells travel in aggregates while covered by a fibrin coating. When these tumor cells reach the capillaries of the target organ, they interact with the endothelial cells through surface glycoproteins, and this interaction slows their transit. Other cells aggregate around these "trapped" cells, thromboplastic events occur, and microthrombi form (Fig 4e,f). Prolonged contact with the endothelium allows tumor cells to extravasate through the basement membrane, where, if the milieu is hospitable, the cells use local growth factors such as transforming growth factor beta or basic fibroblast growth factor to proliferate.[1,2]

Tumors may spread to the head and neck via direct hematogenous, retrograde hematogenous, and/or lymphatic routes.[3] The process we have outlined describes events that occur at the cellular level for the direct hematogenous route, which is the most likely route for infraclavicular tumors to metastasize to the head and neck. For example, in our patient, tumor cells in the renal vein probably reached the vena cava, passed through the pulmonary circulation, and embolized directly to the head and neck via the external carotid artery. In the retrograde route, tumor cells reach the valveless prevertebral and vertebral plexus of veins via anastomoses with the renal veins and are deposited at the skull base by positive pelvic, abdominal, and thoracic pressures. From the skull base, the cells would reach their target via rich, valveless venous communications of the vertebral plexus, cavernous sinus, pharyngeal plexus, and pterygoid plexus. In the lymphatic route, tumor cells may reach the subglottic and supraclavicular lymph nodes through backflow from the thoracic duct.[4]

Renal cell carcinoma is the third most likely infraclavicular tumor to metastasize to the head and neck, preceded only by breast and lung carcinomas.[5,6] Renal cell carcinoma presents between the fifth and seventh decades of life and accounts for 3% of all adult cancers.[7,8] It is two to three times more likely to affect men, and a familial propensity for the disease has been reported. Although most metastases occur early, a solitary metastasis may appear 10 years or more after treatment of the primary tumor. Favored sites of metastases are the lung, bones, and opposite kidney.[8] Metastases to the head and neck occur in 15% of patients; in two thirds, they will be the presenting lesions.[6]

Known head and neck sites of renal cell metastases include the thyroid gland, nose, paranasal sinuses, mandible, skin, gingiva, submandibular gland, temporal bone, palatine tonsil, and tongue.[6,9,10] The calvaria is an extremely unusual site of renal cell metastasis. A metastatic skull lesion may initially manifest as an asymptomatic soft tissue mass, as it did in this patient. A thyroid lesion may initially appear as a painless anterior neck mass. Metastasis to the nose and paranasal sinuses initially appears as nasal obstruction and recurrent epistaxis.[6] Symptoms of sinusitis, exophthalmos, diplopia, ptosis, retro-orbital and frontal pain, facial paresthesia, or anosmia may also be present. Metastasis to the mandible may initially manifest as a loose tooth or lip paresthesia.[9] A metastatic skin lesion appears as a raised, soft, well-circumscribed intracutaneous nodule resembling a pyogenic granuloma or Kaposi's sarcoma.[8]

A CT scan may show a metastatic lesion as a contrast-enhancing, noncalcified soft tissue mass with erosion of the adjacent bony cortex or as an expansile lesion within a paranasal sinus cavity.[6,10] Angiography shows a highly vascular tumor. The histologic appearance varies according to the pathologic variant of renal cell carcinoma. The most common type is the clear cell variant, characterized by polygonal cells containing abundant vacuolated cytoplasm in a highly vascular background.[8] Less common types include the granular cell, sarcomatoid, and collecting duct types. As with our patient, most tumors have mixed features of more than one particular subtype. Frozen sections are unreliable. The differential diagnosis includes oncocytoma, acinic cell carcinoma, thyroid carcinoma, calcifying epithelial odontogenic tumor, and metastatic tumors from other sites.[11]

Metastatic renal cell carcinoma has a poor prognosis and an 80% 5-year mortality rate. Most patients who initially present with metastatic disease, as did our patient, have a median survival of 1 year.[7,8] Patients in whom solitary metastases develop after treatment of primary renal cell carcinoma have a better prognosis, and as many as 30% of these patients may be alive 5 years later. Most authors advocate resection of solitary metastases.[8] Because these tumors are extremely vascular, preoperative embolization is recommended, particularly for sinonasal disease.[10] Traditionally, nephrectomy was reserved for treating localized disease. However, nephrectomy has acquired a new adjuvant role for treating metastatic disease. When performed as part of a multimodality treatment with biologic response modifiers, nephrectomy reduces the tumor burden and may provide autologous cells for treatment with gene-modified tumor vaccines.[12,13]

The current mainstays of treatment for metastatic renal cell carcinoma are biologic response modifiers such as interferon-α, interferon-γ, and interleukin-2,[12-14] which have each been used in various established protocols and yielded individual response rates ranging from 10% to 20%. Most protocols have exclusion criteria for intracranial disease, and therefore, our patient was treated outside of such protocols. Each of these biologic response modifiers has a unique mechanism of action: interferon-α suppresses proliferation of tumor cells, interferon-γ enhances macrophage activation, and interleukin-2 stimulates T-cell growth. Combining these agents in new protocols may be a promising approach.[12] Occasionally, hormonal therapy such as administration of megestrol acetate (a synthetic derivative of progesterone) is used because it improves appetite and has low toxicity. However, except in some isolated anecdotal reports, hormonal therapy has not been shown to be effective. Traditional chemotherapy has uniformly given poor results, but is still used as a last resort for patients treated outside of established protocols. These tumors are radioresistant, and irradiation only has a palliative role for bony metastases.[15]

In summary, although metastases to the head and neck occur infrequently, they should be considered when evaluating any unusual subcutaneous mass in the head and neck. Because metastases have unpredictable timing and clinical behavior, any unusual lesion should be thoroughly evaluated whether or not the patient has a prior history of cancer. Clinicians should consider renal cell carcinoma as a source when evaluating metastases to the thyroid gland, nose, and paranasal sinuses, and the possibility of a metastasis should not be discounted when sites as unlikely as the calvaria are evaluated. Treatment of metastatic renal cell carcinoma is complex, and the optimal regimen for achieving a lasting response without severe toxicity has not been defined.[7] Multidrug immunotherapy has produced promising early results.

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FINITE ELEMENT MODELING OF VOCAL FOLD VIBRATION IN NORMAL PHONATION AND HYPERFUNCTIONAL DYSPHONIA: IMPLICATIONS FOR THE PATHOGENESIS OF VOCAL NODULES

JACK J. JIANG, MD, PHD

CARLOS E. DIAZ, MD

DAVID G. HANSON, MD

CHICAGO, ILLINOIS

A computer model of the vocal fold was developed using finite element modeling technology for studying mechanical stress distribution over vibrating vocal fold tissue. In a simulated normal phonation mode, mechanical stress was found to be lowest at the midpoint of the vocal fold and highest at tendon attachments. However, when other modes predominated, high mechanical stress could occur at the midpoint of the vocal folds. When a vocal fold mass was modeled, high shearing stress occurred at the base of the modeled vocal fold mass, suggesting that the presence of a vocal nodule or polyp is associated with high mechanical stress at the margins of the mass. This finding supports a hypothesis that mechanical intraepithelial stress plays an important role in the development of vocal nodules, polyps, and other lesions that are usually ascribed to hyperfunctional dysphonia.

KEY WORDS — vocal fold model, vocal fold nodule, vocal fold physiology.

INTRODUCTION

In light of the difficulty of measuring glottic vibration in vivo, mathematic models have been helpful in the understanding of vibration of human vocal folds. One-mass and two-mass models[1,2] provided insight into the behavior of the vocal folds, and continuum models[3,4] provided even closer approximation of modeling to the vibratory dynamics of the vocal folds. Finite element analysis (FEA) modeling has been used to study the continuum models of vocal fold vibratory activity.[5,6] This study examined normal and abnormal vibration modes and a few pathologic conditions.

Finite element analysis is a method of mathematically approximating the behavior of structures. The object is divided into elements that define areas within the structure. Finite element analysis allows modeling of vibrations of complex shapes and layered objects. It also provides for definition of particular material properties, and the data can be presented in a pictorial fashion that facilitates interpretation. Finite element analysis has become more available due to the ability to run such models on desktop computers in a relatively short time.

In this study, normal and abnormal vocal fold FEA models were developed based on existing FEA software. The vocal fold vibration modes and the mechanical stress distributions in the vocal folds during normal and abnormal conditions were studied.

METHODS AND MATERIALS

Software I-DEAS VI developed by Structural Dynamics Research Corporation (SDRC) was run via Silicon Graphics hardware in the Civil Engineering and Mechanical Engineering Departments at Northwestern University.

Histologically, the vocal fold consists of three layers: the epithelial cover, the lamina propria, and the muscular body.[7] Functionally, studies of the physical properties of the vocal folds suggest that the epithelium and lamina propria could be effectively represented together as the cover, with the muscle represented as the body.[8] The FEA model used in these studies was based on these assumptions and modeled two layers, designated as the cover and the body. Figure 1A illustrates the vocal fold model in an oblique view. Elements are connected at points referred to as nodes. Each node is defined in the model to have up to 6 degrees of freedom, including displacement in the x, y, and z directions and rotation about the x, y, and z axes. The nodes lateral to the vocal fold body are fixed, because the vocal fold body tissue extends to the rigid internal surface of the thyroid cartilage. By defining forces at the nodes, the displacements and internal tissue mechanical stress can be predicted from the model. Figure 1B demonstrates a vocal fold gross geometry for comparison.

From the Department of Otolaryngology–Head and Neck Surgery, Northwestern University Medical School, Chicago, Illinois. This work was supported by grants P60 DC 94-002-02 and R01 DC00254-11 from the National Institutes of Health/National Institute on Deafness and Other Communication Disorders.

CORRESPONDENCE — Jack J. Jiang, MD, PhD, Dept of Otolaryngology–Head and Neck Surgery, Northwestern University Medical School, Searle Building 12-561, 303 E Chicago Ave, Chicago, IL 60611-3008.

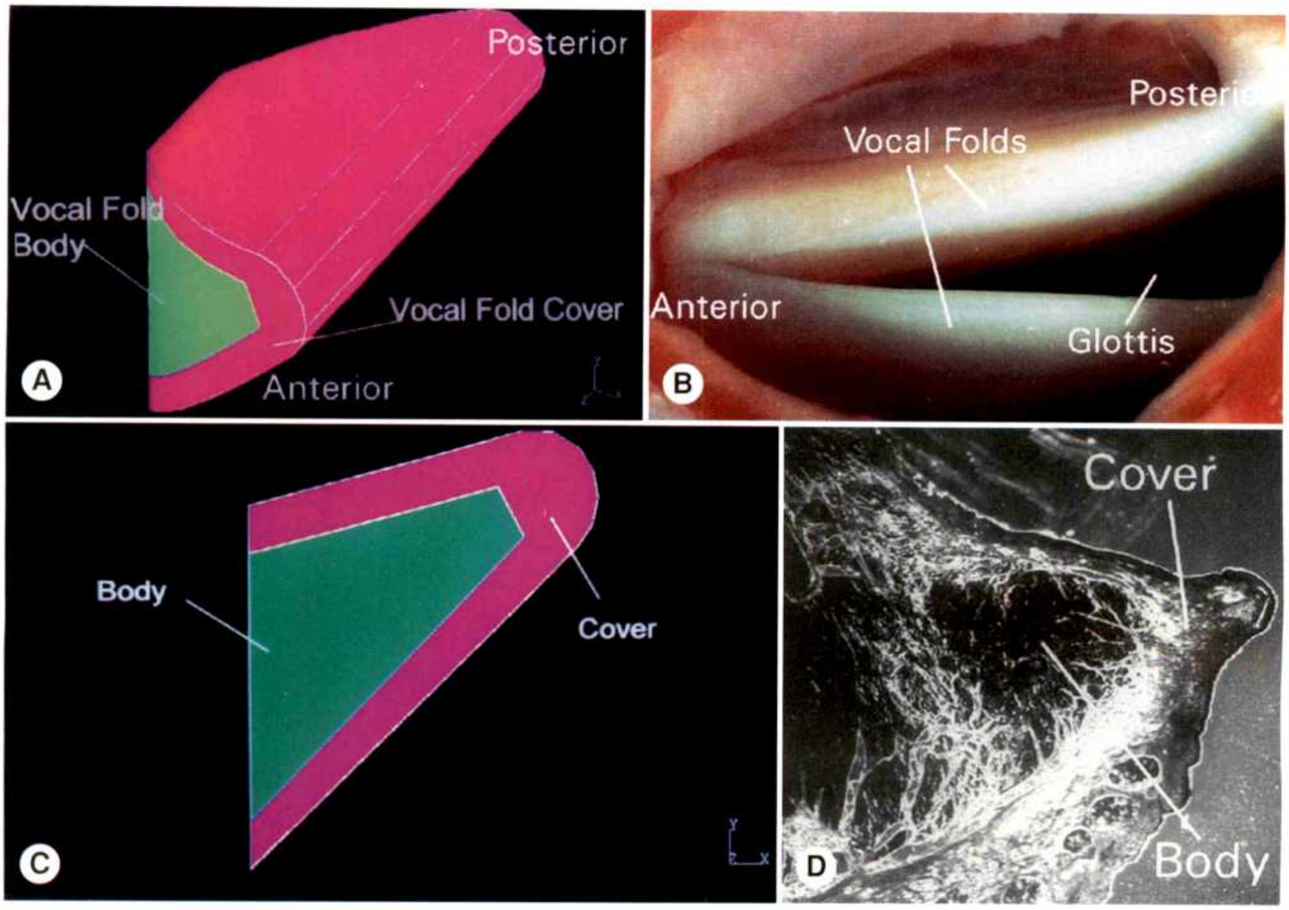

Fig 1. Layers of vocal fold. A) Oblique view of vocal fold model. B) Oblique view of vocal fold gross geometry. C) Cross section of vocal fold model displaying cover and body. D) Coronal section of human vocal fold for comparison (photomicrograph from Dr Malcolm Hast).

Figure 1C shows a cross section of the vocal fold model with body and cover layers, which can be compared to a coronal cross section of a human vocal fold in Fig 1D.

The vocal fold model was created by means of computer-aided drafting. The dimensions used were those of excised human larynges[9] and consist of a length of 17 mm, a height of 10 mm, and a width of 10 mm. The body was assumed to consist of muscle and to have homogeneous properties. The cover was modeled to consist of lamina propria with a uniform thickness of 1.3 mm.[10] Solid elements were chosen so that results could be studied along the depth of the cover. The software generated finite elements from the solid model. The elements of these structures consisted of solid parabolic tetrahedral components. The cover was made up of 303 elements, and the muscle of 40 elements. There were a total of 690 nodes connecting these elements. The nodes representing the laryngeal wall were fixed, while all others were free to move in the x, y, and z directions and rotate about all 3 axes. Thus, each node had 6 degrees of freedom.

Material properties were assigned based on previous measurements of viscoelastic properties of vocal fold tissue. The tissue of the vocal fold was assumed to have isotropic properties. Kakita et al[8] measured viscoelastic properties such as Young's modulus and viscosity for the different layers of canine vocal folds. The lamina propria and muscle were found to have similar properties when the muscle was under no contraction. To simulate an increase in muscle contraction, the stiffness of the muscle was increased by increasing the Young's modulus. Assuming that the histologic characteristics are fairly similar for human vocal folds, we used these values

BIOMECHANICAL PROPERTIES OF VOCAL FOLD TISSUE

Tissue Type	*Young's Modulus (dynes/cm²)*	*Poisson's Ratio*	*Density (g/cm³)*
Vocal fold cover	1	0.3	1.1
Muscle without contraction	1	0.3	1.1
Muscle with 100% contraction	10	0.3	1.1
Vocal nodule	50	0.3	1.1

Information from Kakita et al.[8]

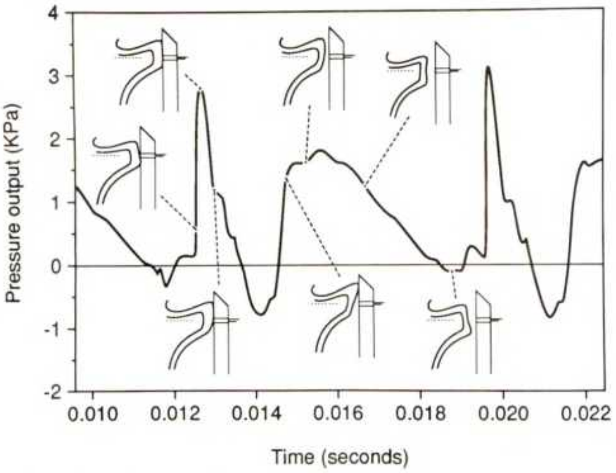

Fig 2. Intraglottal pressure waveform measured from excised larynx. Such pulsed pressures are used to induce vocal fold vibration in finite element analysis model.

for the FEA model; they are presented in the Table.[8]

Tanabe et al[11] measured the damping ratio of excised human larynges. The damping ratio is the major factor determining the mode of vibration of the vocal fold. When the damping ratio is 0, there is no alteration of the amplitude during vibration, while with a ratio of 1, there is no vibration. The value obtained by Tanabe et al, 0.3, was used in the FEA model.

After definition of the mechanical properties, the model was solved to determine the naturally occurring vibrating modes by using a simultaneous vector iteration method. The natural frequency and mode shapes were obtained for each different vibrating mode. Theoretically, the number of vibration modes possible for an object is equal to the number of nodes times the number of degrees of freedom for each node. This study focused on the first 3 low-order vibration modes. Higher-order modes have high-frequency components and decreased vibrating amplitude, and they usually are not excited; thus, it appears that the vibrating pattern of the vocal folds can be explained by the lower-order modes.[6]

When a simulated pressure or force is applied to systems such as those modeled in this study, a modeled vibration results. The variables of direction, frequency, and magnitude imply applied loads that will cause certain vibration modes to dominate. The forced motion that results is similar to the dominant mode shapes. The natural vibrating modes obtained for the FEA model were used to calculate the dynamics of the system. An intraglottal pulsed pressure was used for the dynamic driving pressure. The simulated waveform for the dynamic driving pressure was derived from data obtained from intraglottal measurements on excised larynges,[12] as in Fig 2. The

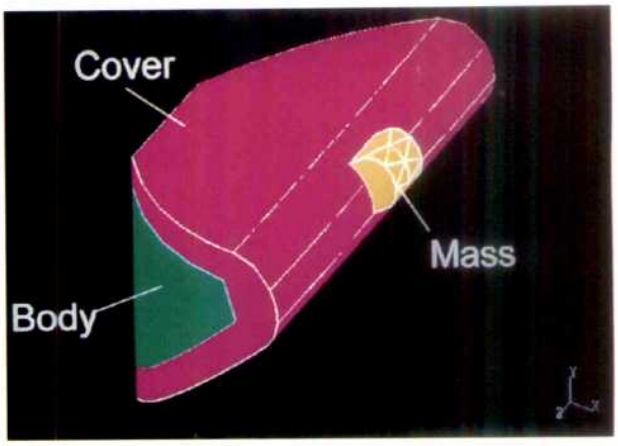

Fig 3. Oblique view of vocal fold model with added $2 \times 2 \times 2$ mm^3 mass simulating nodule.

pressure pulse was applied as a force at 40 points on the vocal fold surface spread throughout the intraglottal area. The direction of the force was approximately perpendicular to the surface of the vocal fold to simulate intraglottal pressure. A frequency of 200 Hz was selected for the driving pressure to approximate a reasonable fundamental frequency of the first mode, which is approximately 200 Hz. The pulsed pressure was modeled as 2.0 kilopascals (kPa).[13]

Normal vocal fold vibration was simulated first. Based on the modes analysis, 3 low-ordered vibratory modes were similar to those seen in in vivo models, and these were studied in detail. Pathologic vocal fold vibration conditions were also simulated. An increased tension dysphonia was simulated by the increase of the stiffness of the vocal fold body from 1×10^6 dynes/cm^2 to 1×10^7 dynes/cm^2. A mass lesion was simulated by adding a $2 \times 2 \times 2$ mm^3 mass at the midpoint of the normal vocal fold model. The Young's modulus of the nodule was specified as 5×10^7 dynes/cm^2. Figure 3 illustrates the vocal fold model with the added mass.

RESULTS

With the structure and mechanical properties of the model specified, the software was used to solve for vibration modes. The first 3 natural vibration mode shapes, frequencies, and mechanical stresses are presented in Fig 4. These were arbitrarily designated as the first, second, and third modes.

The finite element modeling indicated that there were 3 natural modes of vibration that might occur in a modeled vocal fold based on currently understood parameters of the human vocal fold. Figure 4A illustrates that in the first mode, vibration was in both vertical and horizontal directions, with a corresponding frequency of 189 Hz. Figure 4B demonstrates that in the second mode, the anterior and pos-

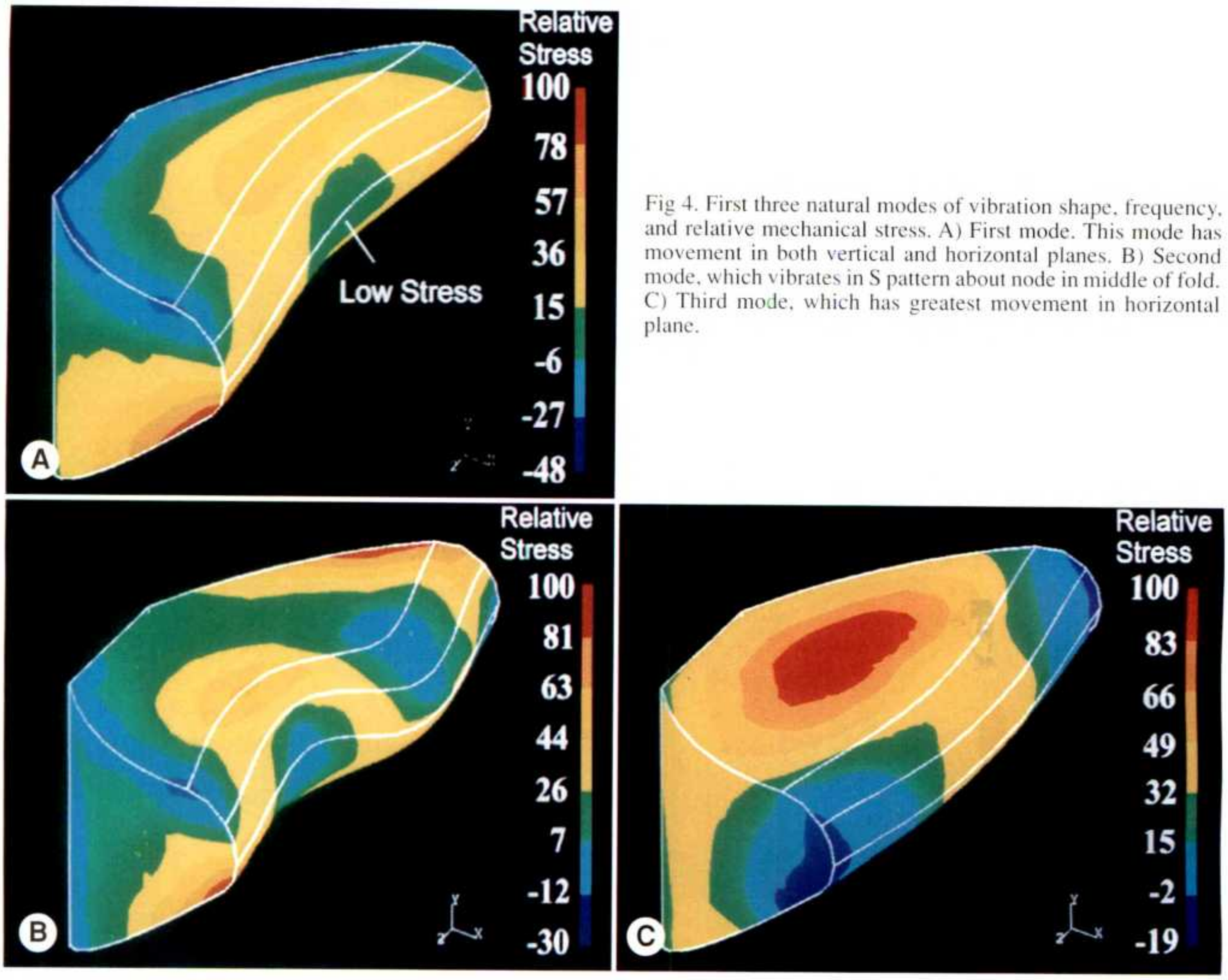

Fig 4. First three natural modes of vibration shape, frequency, and relative mechanical stress. A) First mode. This mode has movement in both vertical and horizontal planes. B) Second mode, which vibrates in S pattern about node in middle of fold. C) Third mode, which has greatest movement in horizontal plane.

terior segments vibrated out of phase with each other about a node at the middle of the vocal fold, with a corresponding natural frequency of vibration of 315 Hz. In Fig 4C, the third natural vibration mode has the greatest amplitude of vibration in a lateral direction at the midportion of the vocal fold, with a natural frequency of vibration of 334 Hz. For the remainder of this discussion, "amplitude" will refer to lateral displacement from midline, where the two vocal folds meet and make contact in the closing phase

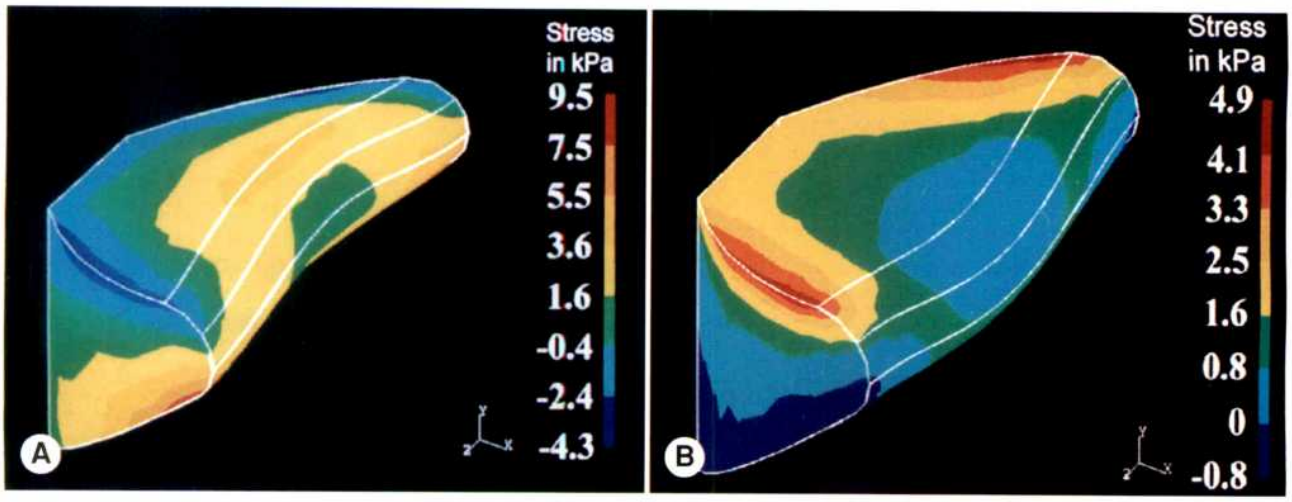

Fig 5. Dynamic shape and mechanical stress in kilopascals at A) beginning of cycle and B) end of cycle, during cycle of vocal fold vibration.

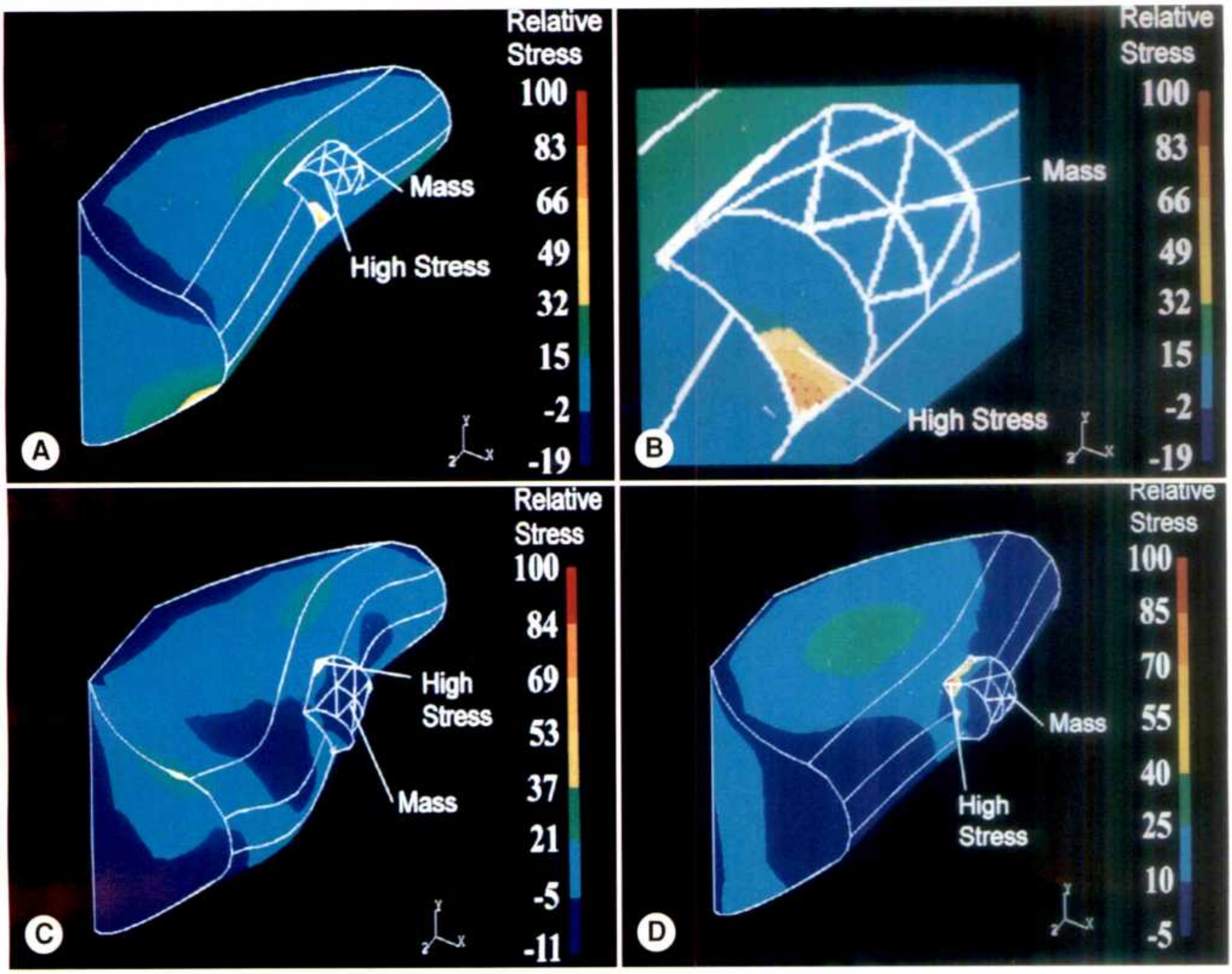

Fig 6. Oblique views of vocal fold model with added nodule in vibration and corresponding relative mechanical stress. A) First mode of vibration. B) Close-up of nodule–vocal fold interface of first mode, demonstrating high relative mechanical stress. C) Second mode of vibration. D) Third mode of vibration.

of the glottal cycle. For the natural vibrating modes described above, the first mode showed high mechanical stress at the edges and top of the vocal fold, with minimal mechanical stress occurring along the medial surface of the fold and minimal mechanical stress at the midpoint. This mode predominated as the most natural vibratory pattern. In the natural first mode of vibration there was relatively little mechanical stress in the midportion of the vocal fold edge (Fig 4A). Mechanical stress prediction in the second and third vibrating modes indicated that high mechanical stress occurred in the cover nodes at the midpoint of the modeled vocal fold (Fig 4B,C).

When an intraglottal pressure was simulated by applying a periodic force to 40 nodes, the resulting forced motion showed a predominance of the first mode, which appears quite similar to the vibration of the normal human glottis. A pulsed pressure of 2 kPa was applied at 200 Hz and the muscle and cover were modeled as being under no tension with a Young's modulus of 1×10^6 dynes/cm^2. Under these conditions, the maximum amplitude was 0.39 mm. Figure 5 shows 2 frames of a dynamic cycle. The dynamic simulation of normal phonation yielded a mechanical stress distribution similar to that predicted for the first natural mode, since this was the mode that predominated.

The model indicates that the vocal fold vibration amplitude is proportional to the driving pressure. When the pulsed pressure was raised from 2 kPa to 3 kPa at 200 Hz, an increase in maximum amplitude of lateral vibratory movement was observed, from 0.39 mm to 0.67 mm.

When the tension of the muscle was increased in the model, an increase in the natural frequencies of vocal fold vibration was observed, from 189 Hz to 294 Hz in the first mode, 315 Hz to 426 Hz in the second mode, and from 334 Hz to 564 Hz in the third mode. The Young's modulus of the cover was maintained at 1×10^6 dynes/cm^2, while the Young's modulus of the muscle was raised to 1×10^7 dynes/cm^2. With increases in the Young's modulus of the modeled muscle, there was a decrease in amplitude from 0.39 mm to 0.25 mm. The stress distributions remained unchanged with these manipulations.

A $2 \times 2 \times 2$ mm^3 mass was added to the modeled vocal fold to simulate a vocal fold nodule. The addition of this small mass was associated with a decrease in natural frequencies of vibration from 189 Hz to 181 Hz in the first mode and no changes in the second mode or third mode. Mechanical stress was predicted to be very high at the base of the nodule. Figure 6A illustrates the first mode of vibration of the vocal fold model with the added nodule and the corresponding stress. Figure 6B is a close-up of the nodule–vocal fold interface demonstrating the high mechanical stress that is predicted to occur during vibration. Figure 6C,D illustrates the vocal fold with the added nodule in the second and third modes of vibration, respectively, and the corresponding mechanical stress distribution.

DISCUSSION

Kaneko et al[14] studied the mechanical properties of vocal folds in which tension was varied and the change in resonant frequency was measured. They found that fundamental frequency increased with increase in tension. With the addition of a mass and a change in the size of the mass, the frequency decreased with increases in mass size. The FEA model reported here behaved very similarly to actual vocal folds in terms of these fundamental characteristics. When the subglottal pressure and muscle tension were varied, the FEA model indicated that the vibration of the midpoint of the modeled vocal fold changed in both amplitude and frequency. The natural frequency of vibration was also decreased by adding a mass in the FEA model. The oscillation pattern induced by a pulsed stimulus that was observed by Kaneko et al was very similar to the motion exhibited by the FEA model that we studied. This correspondence to the mechanical properties of real vocal folds suggests that the FEA model probably represents a useful approximation of the properties of normal vocal fold vibration.

However, the FEA model allows the prediction of several parameters that were previously difficult or impossible to study. Both mechanical stress distribution in the fold and the natural vibration modes are difficult to measure experimentally. In a model that behaves like real vocal folds, parameters can be changed in order to simulate and study pathologic conditions. Mass and stiffness are the major determinants of the natural vibratory frequencies of the vocal folds. Measurements on cancer patients, patients with laryngeal paralysis, and patients with polyps have been performed to assess the effects of changes in mass and stiffness on vocal fold vibration.[14] The increase in natural frequency noted in cancer patients has been attributed to an increase in stiffness caused by the invasive cancer tissue. Patients with laryngeal paralysis demonstrated a decrease in natural frequency that can be attributed to a decrease in stiffness. Polyps have been associated with a decrease in natural frequency that can be attributed to increased mass. These conditions were successfully duplicated by the FEA model. Therefore, the FEA model of vocal fold vibration may be useful for exploring the pathophysiology of various abnormalities that affect vocal fold function.

Vocal fold polyps and vocal fold nodules are common causes of dysphonia and can be of serious consequence for individuals who rely on vocal quality for their livelihood.[15] However, it is not clearly understood how vocal nodules develop. A number of hypotheses have been proposed to explain the cause of vocal nodules and polyps.[16] Several authors[17-19] have implicated mechanical injury in the origin of nodules. Although there is little experimental data to support the theory, nodules are popularly considered to be caused by mechanical trauma. The collision stress between vocal folds during vibration has been measured and found to be correlated with nodule distribution. However, the internal mechanical stress of the vocal fold cover has not been studied previously.

From analysis of these FEA model data, we propose the hypothesis that vocal fold nodule formation occurs as the result of high mechanical stress within the vocal fold epithelium, which causes a localized mechanical trauma. Trauma from mechanical stress may result in acute disruption of epithelial attachments, resulting in hemorrhage and edema in Reinke's space. Chronic, less disruptive mechanical stress may result in tissue growth factors that give rise to thickening of the epithelium. This is analogous to epithelial changes that result from chronic trauma to epithelium at other locations in the body. The data suggest that in normal mode vibration, mechanical stress is low at the midportion of the vocal folds, with the highest mechanical stress occurring at the edge. There is an increase in thickness of the lamina propria from midpoint to edge, with an increase in elastic and collagenous fibers.[10] Thus, in the most natural pattern of modal phonation, the greatest mechanical stress is predicted to occur in areas of the vocal fold that are more resistant to injury, and mechanical stress in the midportion of the vocal fold edge is relatively low. During normal vibration, maximum mechanical stress would occur at points of maximum strength, thus minimizing tissue injury. This would help to explain why trained singers are able to achieve a mode of phonation with which they are able to project great intensity for long periods of time without vocal fold damage.

The second and third natural modes of vibration are associated with prediction of high mechanical stress in the midpoint of the vocal fold. These two modes differ in nature. In the second natural mode of vibration, the anterior and posterior vocal folds vibrate slightly out of phase with each other. This mode of vibration may correspond to the glottic configuration that has been described in the larynges of patients with hyperfunctional dysphonia as the muscular tension dysphonia posture.[20] In these patients, the anterior portions of the vocal folds are approximated, while the posterior portions of the vocal folds are less approximated, resulting in a Y-shaped configuration. When there is a difference in vocal fold approximation, we would predict that the vibration of the vocal fold cover may differ in phase between the two segments. When this is the case, the vibratory mode may approximate the second natural vibration mode predicted for the FEA model. The model predicts that high mechanical stress would occur at the segment between the vibratory segments. Clinical correlation indicates that this is the location at which nodules develop.

A combination of the first and third natural modes of phonation is associated with increased horizontal and vertical amplitudes of vibration. We hypothesize that this mode may correspond with a very high flow rate, as may be present with shouting. The FEA model predicts high mechanical stress at the midportion of the vocal fold for this mode of phonation, and this correlates with the site of acute injury most often seen after acute vocal abuse injuries.

Some theories about the development of vocal fold nodules hypothesize that the injury is caused by the vocal folds colliding with excessive adduction. The FEA model suggests that excessive collision stress due to the amplitude of vocal fold vibration may not be the only factor in causing injury. The vocal fold vibration mode may be more important. If pathologic variation in vocal fold tension, flow, or pressure causes vibration to predominately take the form of a natural vibratory mode other than the first natural mode, high mechanical stress is predicted at the very sites in which we clinically identify both acute and chronic injury. It is therefore likely that intraepithelial mechanical stress could be the primary factor that contributes to the formation of acute vocal fold polyps and chronic nodules. An abnormal vibration mode may be more damaging to the vocal fold than high intensity of vibration.

In addition, the FEA model also suggests that once a nodule-like mass is present on the free margin of the vocal fold, then high mechanical stress occurs at the base of the nodule during vibration in all modes. This may imply that once a localized edema or nodule occurs, any further vocal fold vibration will produce high stress at the base of the vocal fold nodule. Such stress would predictably cause further mechanical stress damage with an increase in mass size and progressively greater effect. This hypothesis supports the observation that voice rest is useful in achieving reduction of acute vocal fold injuries.

More information is needed regarding the mechanical properties of the interface between the lamina propria and muscle to allow development of better models. Future studies should include the modeling of collision stress between vocal folds and the interaction between vocal folds during vibration. Attempts should be made to simulate in vivo conditions more closely by using material properties that are more similar to those of actual vocal folds.

CONCLUSION

An FEA model of vocal fold vibration, based on known physical parameters of vocal folds, was used to predict natural modes of vibration and mechanical stress in the vibrating vocal fold cover. The data correlated well with modeling of vocal fold vibration derived from excised larynges. In addition, variations in parameters such as tension and mass demonstrated effects on frequency and amplitude of vibration that we would predict from previous models. In the predominant natural mode of vibration, mechanical stress remains relatively low in the midportion of the vocal fold, and maximal mechanical stress is at the anterior and posterior margin attachments. However, in secondary natural modes of phonation that may correlate with hyperfunctional dysphonia modes, there is a peak mechanical stress located in the midportion of the membranous folds, at the site in which vocal fold polyps and nodules are noted to develop. In addition, the base of vocal fold nodules is predicted to have much higher mechanical stress during vibration than surrounding tissue, even in the primary natural mode of vibration. Therefore, the hypothesis that mechanical stress plays an important role in the development of hyperfunctional vocal fold lesions is supported by these modeling studies. Since mechanical stress appears to play a role in the development of blisters and callus formation from epithelial damage in other portions of the human epithelium, this hypothesis appears to be reasonable from the aspect of known epithelial response to repeated trauma.

Finite element modeling of vocal fold vibration appears to be a useful tool for predicting the pathophysiology of abnormal phonatory conditions and may have applications for the understanding of abnormal vocal fold vibration in other disorders.

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INTRACELLULAR CALCIUM CHANGES AND CHEMOSENSITIVITIES OF HUMAN EPIDERMOID CARCINOMA CELL LINES AFTER EXPOSURE TO CISPLATIN

TAKUYA TACHIKAWA, MD HIROBUMI KUMAZAWA, MD
YOSHIO HORI, MD NARINOBU HARADA, MD SHIN-ICHI SAI, MD
TOSHIO YAMASHITA, MD SUSUMU YODOSAWA, MD KEIJI KAWAMOTO, MD
OSAKA, JAPAN

In order to elucidate the mechanisms of cisplatin (cis-diamminedichloroplatinum; CDDP)–resistant tumor cells, we previously established a CDDP-resistant KB cell line (KBrc cells) from a parental KB cell line derived from epidermoid carcinoma (KB cells). The KBrc cells were resistant to 5 kinds of platinum (Pt) drugs. Intracellular Pt concentrations in KBrc cells were lower than in KB cells. Decrease of intracellular Pt concentrations was one of the CDDP-resistant mechanisms. When we measured changes of intracellular calcium ion concentration ($[Ca^{2+}]i$) during exposure to high-dose CDDP, a sustained elevation of the $[Ca^{2+}]i$ level was observed in the KB cells. These results suggest that the mechanisms underlying CDDP resistance involve changes in calcium channels and an alteration of calcium homeostasis in the tumor cell line.

KEY WORDS — CDDP-resistant KB cells, intracellular free calcium ion concentration, intracellular platinum concentration, KB cells.

INTRODUCTION

Cisplatin (cis-diamminedichloroplatinum; CDDP) is one of the most potent and useful antitumor agents for the treatment of malignant solid tumor.[1] The cytotoxicity of this drug is known to include an inhibition of DNA synthesis.[2] The occurrence of resistance to CDDP in tumor cells is an important problem in its clinical application.[3] To investigate the mechanism of underlying CDDP resistance in tumor cells, numerous studies have reported the establishment of CDDP-resistant sublines.[4-8] The development and study of CDDP-resistant cells has demonstrated that the CDDP-resistant mechanism is multifactorial, including a decreased accumulation of CDDP,[5] an increased level of glutathione,[6] enhanced repair of damaged DNA,[7] and alterations in signal transduction pathways.[8] In addition, intracellular calcium ion is a key regulatory component of cellular metabolism and the signal transduction pathway,[9] and recent studies have suggested that calcium ion (Ca^{2+}) may play an important role in cell death and toxic injury.[10-12]

To elucidate the role of Ca^{2+} in the mechanisms underlying CDDP resistance in head and neck tumor cell lines, we investigated the mobilization of Ca^{2+} in an epidermoid carcinoma cell line (KB cells)[13] and a CDDP-resistant KB cell line previously established in our laboratory (KBrc cells).[14]

MATERIAL AND METHODS

Drugs. Antitumor drugs were obtained from the following suppliers: CDDP, cis-1,1,-cyclobutanedicarboxylato (2R)-2-methyl-1,4-butanediamine platinum (II) (NK-121), and pirarubicin (THP-ADR), Nihon Kayaku Co Ltd, Tokyo, Japan; (–)-(R)-2-aminomethylpyrrolide (1,1-cyclobutanedicarboxylate) platinum (II) (DWA2114R), Cyugai Pharmaceutical, Tokyo; cis-diammine(glycolate)-platinum (II) (254-S), Shionogi Pharmaceutical, Osaka, Japan; cis-diammine-1,1-cyclobutane dicarboxylate platinum (II) (CBDCA), Bristol-Myers Squibb, Tokyo; Adriamycin (ADR), Kyowa Hakko Co Ltd, Tokyo; and aclarubicin (ACR), Sanraku Ocean, Tokyo. NK121, DWA2114R, and 254-S are new platinum (Pt) analog drugs developed in Japan[15-17] (Fig 1). The drugs were dissolved with RPMI 1640 medium before being used in the experiments.

The standard solution for intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) measurements was composed of sodium chloride 150 mmol/L, potassium chloride 5 mmol/L, dipotassium hydrogen phosphate 0.2 mmol/L, potassium dihydrogen phosphate 0.8 mmol/L, magnesium chloride 1 mmol/L, calcium chloride 1 mmol/L, and glucose 5 mmol/L at pH 7.4 (Nacalai

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From the Department of Otolaryngology (Tachikawa, Kumazawa, Hori, Harada, Sai, Yamashita), the First Department of Pathology (Yodosawa), and the Department of Neurosurgery (Kawamoto), Kansai Medical University, Osaka, Japan. This work was supported in part by a grant from the Ministry of Education, Science and Culture of Japan (number 05771369).

CORRESPONDENCE — Takuya Tachikawa, MD, Dept of Otolaryngology, Kansai Medical University, Fumizono-cho-10-15, Osaka 570, Japan.

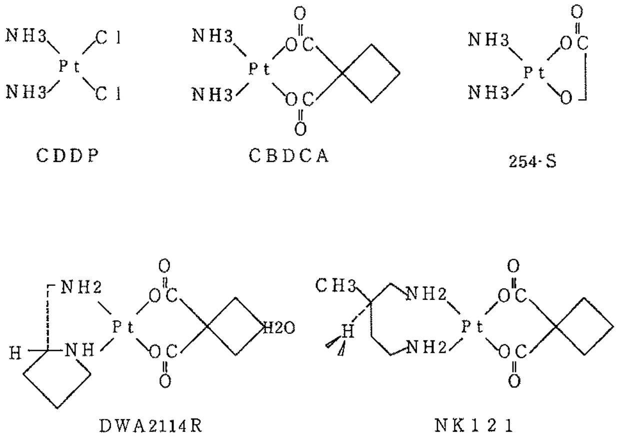

Fig 1. Structures of CDDP and CDDP analog drugs. 254-S, DWA2114R, and NK121 were new platinum derivatives developed in Japan. CDDP — cis-diamminedichloroplatinum (II); CBDCA — cis-diammine-1,1-cyclobutane dicarboxylate platinum (II); 254-S — cis-diammine (glycolate)-platinum (II); DWA2114R — (–)-(R)-2-aminomethylpyrrolide (1,1-cyclobutanedicarboxylate) platinum (II); NK121 — cis-1,1,-cyclobutanedicarboxylato (2R)-2-methyl-1,4-butanediamine platinum (II).

Tesque, Kyoto, Japan). For experiments done under Ca^{2+}-free conditions, the standard solution was prepared by omitting the calcium chloride and adding 1 mmol/L ethylene glycol-bis (β-aminoethylether) NNN'N'-tetraacetic acid (EGTA; Sigma Chemical, USA). Lanthanum chloride (LaCl3; Nacalai Tesque) was used for the calcium channel blocker.

Cell Lines and Culture. The parental cell line (KB cells) was obtained from Dainippon Pharmaceutical Co Ltd (Tokyo) and was derived from human epidermoid carcinoma.[12] The CDDP-resistant cell line, KBrc cells, was previously established by exposure of KB cells to stepwise-increasing CDDP concentrations (range 0.01 to 1.0 μg/mL) and selected by limiting dilution. The KBrc cells were 2.0-fold resistant to CDDP compared with the parental KB cells, on the basis of the 50% inhibition concentration in trypan blue dye exclusion with continuous drug exposure.[14] The KB cells were cultured in RPMI 1640 medium (Nissui Pharmaceutical Co Ltd, Tokyo) supplemented with 10% heat-inactivated fetal bovine serum, 50 μg/mL streptomycin, 50 IU/mL penicillin, and 10 mmol/L L-glutamine (Flow Laboratories, Australia). The KBrc cells were cultured in the former medium with 1.0 μg/mL CDDP. Both cell lines were grown at 37°C in a humidified 5% carbon dioxide atmosphere incubator, and were used for the experiments when they were in logarithmic growth phase. Also, KBrc cells were cultured with RPMI 1640 medium without CDDP for 3 days before the experiment.

Measurement of Chemosensitivities in Cell Lines. We performed chemosensitivity testing as previously reported.[18] In brief, 2,000 cells per well were cultured in 96-well multiplates (Corning, USA) in RPMI 1640 medium with or without drugs at 37°C in a 5% carbon dioxide humidified incubator for 72 hours. Then, the medium in each well was removed. After addition of 20 μL of 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyl-2H tetrazolium bromide (MTT) solution containing 0.4% MTT (Dojindo Chemical Co Ltd, Kumamoto, Japan) and 0.1 mol/L succinic acid disodium salt (Nacalai Tesque) to the chambers, the cells were incubated for 3 hours. Insoluble formazan salts, converted from the soluble MTT by viable cell precipitates as crystal, were dissolved in 150 μL of dimethylsulfoxide (Sigma Chemical, USA) and quantified by measurement of the optical density (OD) using a microplate reader (Titertek Multiskan MCC 340, USA) with dual wavelengths consisting of a measurement wavelength (540 nm) and a reference wavelength (630 nm). The chemosensitivities of the antitumor drugs were evaluated by comparison of the OD 50, which was the drug concentration causing a 50% reduction of the OD in the control group without drugs.

Measurement of Intracellular Platinum Concentrations. Ten million cells per flask (75 cm^2; Corning) were cultured for 24 hours. The cells were then exposed to 10 μg/mL or 100 μg/mL CDDP or CBDCA for 6 hours. The cells were then harvested with trypsin and rinsed with cold phosphate-buffered saline 3 times. The measurement of intracellular Pt concentrations was performed by a modification of the method of LeRoy et al.[19] In brief, the cells were hydrolyzed in nitric acid, and the Pt contents of the cells were chelated with sodium hydroxide. The Pt was separated from the chelated compounds with chloroform. The intracellular Pt concentrations were measured with a spectrophotometer at a wavelength of 265.9 nm.

Determination of $[Ca^{2+}]i$ by Fura-2. Measurements of $[Ca^{2+}]i$ were made following a protocol similar to those we previously reported.[20-22] In brief, individual Fura-2–loaded tumor cells were examined with an X40 Fluor objective (Nikon, Tokyo) on the stage of an inverted microscope (TMD-2, Nikon). The microscope system consisted of an inverted microscope equipped with high-resolution differential interference contrast imaging. For the excitation light, a 75-W xenon lamp was passed through interference and neutral-density filters in which the half bandwidth was 10 nm. After passing through these filters, the excitation light was reflected by a dichroic mirror with a half-pass wavelength of 400 nm. The emitted light obtained from the Fura-2 fluorescence of an isolated cell, passed through the same dichroic mirror, a 20-nm band pass, and a 510-nm

TABLE 1. FIFTY PERCENT OPTICAL DENSITIES FOLLOWING TREATMENTS WITH SEVERAL ANTITUMOR DRUGS

Drug	*KB Cells (mg/mL)*	*KBrc Cells (mg/mL)*	*RR*
CDDP	$5.41 \pm 1.02 \times 10^{-4}$	$1.02 \pm 0.33 \times 10^{-3}$	1.9*
CBDCA	$1.08 \pm 0.31 \times 10^{-2}$	$1.72 \pm 0.93 \times 10^{-2}$	1.6*
254-S	$3.33 \pm 1.72 \times 10^{-3}$	$4.54 \pm 1.43 \times 10^{-3}$	1.4*
NK121	$0.98 \pm 0.33 \times 10^{-2}$	$2.45 \pm 1.64 \times 10^{-2}$	2.5*
DWA2114R	$4.68 \pm 1.98 \times 10^{-2}$	$6.28 \pm 1.27 \times 10^{-2}$	1.3*
ADR	$1.35 \pm 0.88 \times 10^{-5}$	$1.55 \pm 0.78 \times 10^{-5}$	1.1
THP-ADR	$4.20 \pm 0.97 \times 10^{-6}$	$4.70 \pm 1.04 \times 10^{-6}$	1.1
ACR	$1.15 \pm 0.97 \times 10^{-5}$	$1.54 \pm 1.31 \times 10^{-5}$	1.3

Chemosensitivities of cells to drugs were evaluated by comparing 50% optical density. CDDP-resistant KB (KBrc) cells were 1.9-fold more resistant to CDDP and 1.3- to 2.5-fold more resistant to other platinum drugs than parental KB cells.

CDDP — cis-diamminedichloroplatinum (II); CBDCA — cis-diammine-1,1-cyclobutane dicarboxylate platinum (II); 254-S — cis-diammine(glycolate)-platinum (II); NK121 — cis-1,1-cyclobutanedicarboxylato (2R)-2-methyl-1,4-butanediamine platinum (II); DWA2114R — (–)-(R)-2-aminomethylpyrrolide (1,1-cyclobutanedicarboxylate) platinum (II); ADR — Adriamycin; THP-ADR — pirarubicin; ACR — aclarubicin; RR — relative resistance.

*Significant difference ($p < .05$).

TABLE 2. INTRACELLULAR PLATINUM CONCENTRATIONS IN MICROGRAMS PER 10^7 CELLS

	KB Cells	*KBrc Cells*	*% Control Uptake*
CDDP 10 μg/mL	0.75	0.55	73
CDDP 100 μg/mL	5.89	3.75	61
CBDCA 10 μg/mL	0.34	0.16	47
CBDCA 100 μg/mL	1.47	0.85	58

Intracellular platinum (Pt) concentrations in KBrc cells were lower than those in KB cells. Intracellular Pt concentrations were reduced to 73% and 61% compared with KB cells exposed to 10 and 100 μg/mL CDDP, respectively. Intracellular Pt concentrations in KBrc cells were reduced to 47% and 58% compared with KB cells exposed to 10 and 100 μg/mL CBDCA, respectively.

barrier filter, was then focused on a low-light silicon intensifier target camera and was then fed into the computer analysis system (Argus 100/Ca, Hamamatsu Photonics KK, Hamamatsu, Japan). The Fura-2 emission intensity at 2 excitation wavelengths, 340 and 380 nm, was obtained, and the $[Ca^{2+}]i$ was calculated.

Measurement of $[Ca^{2+}]i$ Exposed to CDDP. After KB and KBrc cells were cultured in Petriperm with RPMI 1640 for 24 hours, both cell lines were loaded with 5 μmol/L Fura-2 for 30 minutes at room temperature. The cells were washed in phosphate-buffered saline and added to 2 mL fresh standard solution. The Petriperm was then mounted on the stage of a Nikon TMD inverted microscope. The solution in the Petriperm was continuously circulated at 1 mL/min by a Microperpex pump (LKB 2132, Pharmacia LKB Biotechnology, AB). Each solution was adjusted to an osmolarity of 300 mOsm. All experiments were performed at room temperature (22°C to 25°C).

Viabilities of Tumor Cells Exposed to CDDP. After the experiments on $[Ca^{2+}]i$ measurement, the cells in the Petriperm were cultured with the medium for 6 or 12 hours. The cell viabilities were measured by trypan blue dye exclusion.

Two thousand cells per well in 96-well multiplates were cultured in RPMI 1640 medium in the incubator. After cultivation for 24 hours, the cells in the multiplates were exposed to 500 μg/mL CDDP with 1 mmol/L Ca^{2+} in the standard solution for 2 minutes. After removal of the drugs from the plates, the cells in the plates were rinsed with phosphate-buffered saline twice, and the cells were cultured in RPMI 1640 medium for 12 hours. The OD was then measured in the same manner as in the chemosensitivity testing.

Statistical Analysis. Comparisons between data from the KB and KBrc cells were performed with Student's *t*-test. A p value of less than .05 was considered to have statistical significance.

RESULTS

Measurement of Chemosensitivity in Cell Lines. The chemosensitivities of the cells to the drugs were evaluated in terms of the OD 50. The KBrc cells were 1.9-fold resistant to CDDP and 1.3- to 2.5-fold resistant to other Pt drugs as compared to parental KB cells (Table 1).

Measurement of Intracellular Platinum Concentrations. The intracellular Pt concentrations in the KBrc cells were much lower than those in the KB cells when CDDP or CBDCA was exposed to the cell chambers at a concentration of 10 or 100 μg/mL. The intracellular Pt concentrations in KB cells after incubation with 10 and 100 μg/mL of CDDP were 0.75 and 5.89 μg/10^7 cells, respectively. The intracellular Pt concentrations in the KBrc cells after incubation with 10 and 100 μg/mL of CDDP were 0.55 and 3.75 μg/10^7 cells, respectively. The relative Pt concentrations in the KBrc cells, when compared to KB cells, were 73% and 61% after incubation with 10 and 100 μg/mL CDDP, respectively. The intracellular Pt concentrations in KB cells exposed to 10 and 100 μg/mL CBDCA were 0.34 and 1.47 μg/10^7 cells, respectively. The intracellular Pt concentrations in the KBrc cells exposed to 10 and 100 μg/mL CBDCA were 0.16 and 0.85 μg/10^7 cells, respectively. The relative values in KBrc cells after incubation with 10 and 100 μg/mL CBDCA were 47% and 58%, respectively, compared with KB cells (Table 2). We tried to measure intracellular Pt con-

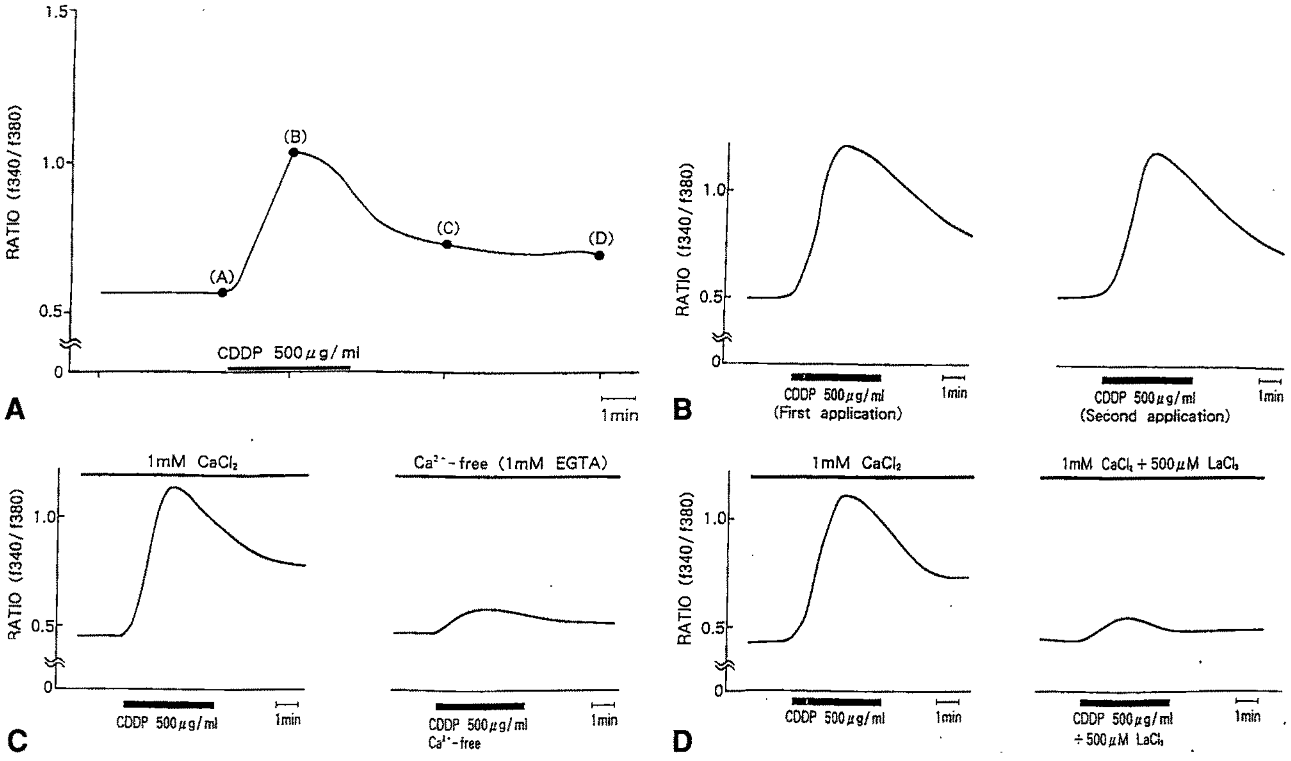

Fig 2. Time course of intracellular calcium ion concentration ([Ca^{2+}]i) changes in cells exposed to CDDP. f340 — fluorescence intensity at 340 nm wavelength, f380 — fluorescence intensity at 380 nm wavelength. A) Time course of [Ca^{2+}]i change in typical KB cell following application of 500 μg/mL CDDP in presence of extracellular 1 mmol/L calcium ion (Ca^{2+}) with circulation pump, in Fig 3 at 4 points indicated in pseudocolor imaging. B) Time course of reproducible repeated increases in [Ca^{2+}]i following application of 500 μg/mL CDDP in typical KB cell. Second application of CDDP was performed 15 minutes after first application. C) Time course of [Ca^{2+}]i kinetics following application of 500 μg/mL CDDP in presence of extracellular 1 mmol/L Ca^{2+} and in absence of extracellular Ca^{2+}, respectively. Application of CDDP in Ca^{2+}-free medium only slightly increased [Ca^{2+}]i in KB cell. Interval between two measurements was 15 minutes. CaCl2 — calcium chloride, EGTA — ethylene glycol-bis (β-aminoethylether) NNN'N'-tetraacetic acid. D) Time course of [Ca^{2+}]i change upon exposure to 500 μg/mL CDDP alone and 500 μg/mL CDDP with 500 μmol/L lanthanum chloride (LaCl3) in typical cell, respectively. Interval between two measurements was 15 minutes. Increase in [Ca^{2+}]i in KB cell during exposure to CDDP was significantly suppressed by LaCl3.

centrations in cells exposed to 500 μg/mL of CDDP for 6 hours, which was a very high dose; since most of the cells exposed to 500 μg/mL of CDDP were damaged, we could not measure the intracellular Pt concentrations.

Effect of CDDP on [Ca^{2+}]i. The effects of CDDP on the [Ca^{2+}]i were studied in KB cells. In response to 500 μg/mL of CDDP, the [Ca^{2+}]i in the KB cells increased to the peak value in less than 2 minutes, and then decreased toward the resting level following washout of the CDDP from the external solution. This is illustrated in a KB cell in Figs 2A and 3. In addition, the amplitude of the [Ca^{2+}]i increase induced by CDDP was essentially the same when the measurement was repeated. This is illustrated in Fig 2B in a sample KB cell. To investigate the mechanisms of CDDP-induced [Ca^{2+}]i changes, we performed measurements of [Ca^{2+}]i changes during exposure to 500 μg/mL of CDDP with and without extracellular Ca^{2+} (1 mmol/L). In comparison with the results obtained in the presence of extracellular Ca^{2+} (1 mmol/L calcium chloride), the increase of CDDP-induced [Ca^{2+}]i in the absence of extracellular Ca^{2+} (addition of 1 mmol/L EGTA) was smaller but still significant in 11 of 14 cells. No further increase in [Ca^{2+}]i was observed in the other 3 cells. This is illustrated in Fig 2C in a sample KB cell. To study the pharmacology of these Ca^{2+} influx pathways, the effects of a calcium channel blocker on the CDDP-induced Ca^{2+} influx was also examined. When 100 μmol/L LaCl3 was added to the external solution, the [Ca^{2+}]i increase induced by 500 μg/mL of CDDP was not changed compared with that in solution without LaCl3 (data not shown). In contrast, when 500 μmol/L of LaCl3 was added to the external solution, the [Ca^{2+}]i increase induced by 500 μg/mL of CDDP was significantly attenuated as compared with the control group without LaCl3. The peak of [Ca^{2+}]i in the presence of 500 μmol/L of LaCl3 was reduced to 12% of the peak [Ca^{2+}]i in the first application of CDDP (n = 10). This is illustrated in Fig 2D in a

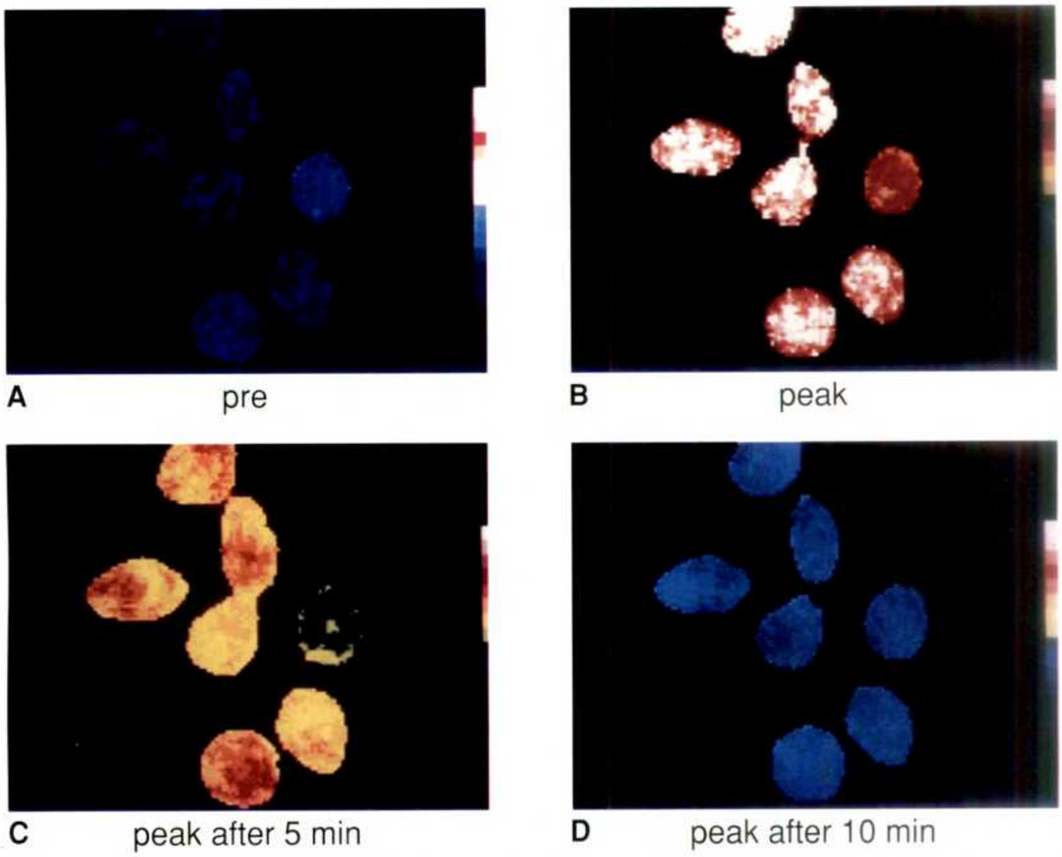

Fig 3. Pseudocolor imaging of intracellular Ca^{2+} distribution in KB cells subsequently recorded in case of exposure to 500 μg/mL CDDP with circulation pump at 4 points (A-D) indicated in Fig 2A. A) $[Ca^{2+}]i$ in control. B) $[Ca^{2+}]i$ in peak. C) $[Ca^{2+}]i$ 5 minutes after peak in $[Ca^{2+}]i$, application of CDDP. D) $[Ca^{2+}]i$ 10 minutes after peak in $[Ca^{2+}]i$, application of CDDP. Color gradation bar is scale bar of $[Ca^{2+}]i$. White color means higher $[Ca^{2+}]i$; blue color means lower $[Ca^{2+}]i$.

sample KB cell. After washout of 500 μmol/L of $LaCl_3$, the $[Ca^{2+}]i$ increases in response to CDDP recovered to a normal pattern (data not shown).

These CDDP-induced $[Ca^{2+}]i$ changes were dose-dependent in regard to CDDP concentrations (range of CDDP, 125 to 400 μg/mL; Table 3). In the case of exposure to 0.1 or 1.0 μg/mL of CDDP for 24 hours, the $[Ca^{2+}]i$ in KB cells was slightly decreased, but there was no significant difference compared with $[Ca^{2+}]i$ in the control group (Table 4). In contrast, after exposure to 10 or 20 μg/mL of CDDP for 24 hours, the $[Ca^{2+}]i$ in KB cells increased to 0.59 ± 0.08 and 0.77 ± 0.15, respectively. When KB cells were exposed to 500 μg/mL of CDDP for 15 minutes, a continuous elevation of the $[Ca^{2+}]i$ was observed (Table 5).

TABLE 3. CHANGES IN INTRACELLULAR CALCIUM ION CONCENTRATION AFTER EXPOSURE TO SEVERAL CONCENTRATIONS OF CDDP WITH CIRCULATION PUMP

	$[Ca^{2+}]i$ in Control	*$[Ca^{2+}]i$ in Peak*
125 μg/mL	0.52 ± 0.02	0.58 ± 0.02*
200 μg/mL	0.53 ± 0.03	0.65 ± 0.03*
300 μg/mL	0.42 ± 0.03	0.67 ± 0.02*
400 μg/mL	0.47 ± 0.03	0.88 ± 0.11*

Values represent means ± SD calculated from 3 independent experiments.

*$[Ca^{2+}]i$ concentration in peak was higher than that in control ($p < .05$).

Viability of Cells Exposed to CDDP With or Without Ca^{2+}. We investigated whether or not extracellular Ca^{2+} affects the cell viability of KB cells exposed to high-dose CDDP. After measurements of

TABLE 4. CHANGES IN INTRACELLULAR CALCIUM ION CONCENTRATION AFTER EXPOSURE TO SEVERAL CONCENTRATIONS OF CDDP FOR 24 HOURS

	$[Ca^{2+}]i$ in Control	*$[Ca^{2+}]i$ in Drug Exposure*
0.1 μg/mL	0.49 ± 0.04	0.45 ± 0.03*
1.0 μg/mL	0.47 ± 0.04	0.42 ± 0.03*
10 μg/mL	0.48 ± 0.07	0.59 ± 0.08†
20 μg/mL	0.48 ± 0.07	0.77 ± 0.15†

Values represent means ± SD calculated from 3 independent experiments.

*No significant difference was observed compared with $[Ca^{2+}]i$ in control ($p > .05$).

†Significant difference was observed compared with $[Ca^{2+}]i$ in control ($p < .05$).

TABLE 5. CHANGES IN INTRACELLULAR CALCIUM ION CONCENTRATION AFTER CONTINUOUS EXPOSURE TO 500 μG/ML CDDP

Pre-exposure	*2 min*	*10 min*	*15 min*
0.43 ± 0.03	1.18 ± 0.11	1.56 ± 0.15	1.94 ± 0.21

Values represent means ± SD calculated from 6 independent experiments.

$[Ca^{2+}]$i exposed to 500 μg/mL of CDDP with or without extracellular 1 mmol/L Ca^{2+}, the KB cells in the Petriperm were reincubated in the RPMI 1640 medium for 6 or 12 hours. In cases without Ca^{2+}, the cell viabilities of the KB cells were 53.8% ± 9.1% and 27.6% ± 0.6%, respectively. The cell viabilities of KB cells exposed to CDDP with 1 mmol/L Ca^{2+} were 37.0% ± 4.1% and 14.6% ± 2.2%, respectively. These results indicate that a $[Ca^{2+}]$i increase affects cell viabilities in KB cells besides the DNA-Pt complex. These results are shown in Table 6.

Comparison of $[Ca^{2+}]$i Changes Between KB and KBrc Cells. We also compared the $[Ca^{2+}]$i changes in both cell lines exposed to 500 μg/mL of CDDP. The CDDP elevated the $[Ca^{2+}]$i in both cell lines, and the early transient elevation in $[Ca^{2+}]$i was relatively rapid. In contrast, with washout of CDDP from the chamber, the levels of $[Ca^{2+}]$i gradually returned toward the initial level. The peak of $[Ca^{2+}]$i in the KB cells during exposure to CDDP was higher than that in the KBrc cells. The $[Ca^{2+}]$i returned to the resting level more slowly in the KB cells than in the KBrc cells. In addition, the sustained elevation of $[Ca^{2+}]$i in the KB cells was higher than that in the KBrc cells. However, the basal $[Ca^{2+}]$i in the resting state was not significantly different between the KB and KBrc cells (Fig 4). These elevations of the $[Ca^{2+}]$i in both cell lines were mainly due to influx of extracellular Ca^{2+} through calcium channels.

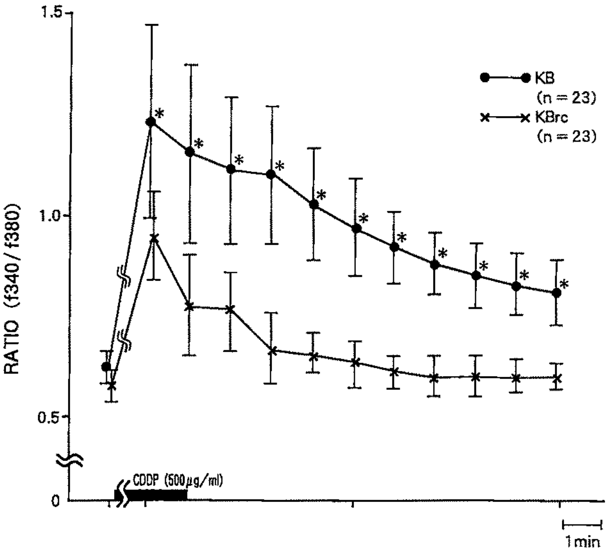

Fig 4. Comparison of $[Ca^{2+}]$i changes between KB and KBrc cells. Changes in $[Ca^{2+}]$i concentration in KB cells were more rapid than those in KBrc cells, and sustained peak level of $[Ca^{2+}]$i in KB cells was higher than level in KBrc cells. * — $[Ca^{2+}]$i concentration in KB cells was significantly higher than that in KBrc cells.

Viabilities of Both Cell Lines Exposed to High-Dose CDDP. After measurements of $[Ca^{2+}]$i changes in cells exposed to 500 μg/mL of CDDP with 1 mmol/L Ca^{2+}, the cells in the Petriperm were cultured with RPMI 1640 for 6 or 12 hours. The cell viability of KB cells was 36.2% ± 5.4% after 6 hours and 14.9% ± 2.4% after 12 hours. In the KBrc cells, the cell viability with 1 mmol/L Ca^{2+} was 61.9% ± 4.8% after 6 hours and 20.7% ± 0.5% after 12 hours. A significant difference in the cell viabilities was observed between the KB and the KBrc cells (Table 7).

TABLE 6. CELL VIABILITIES OF KB CELLS 6 AND 12 HOURS FOLLOWING EXPOSURE TO 500 μG/ML CDDP WITH OR WITHOUT 1 MMOL/L CALCIUM ION IN STANDARD SOLUTION

	6 h	*12 h*
$[Ca^{2+}]$ 1 mmol/L (+)	37.0% ± 4.1%*	14.6% ± 2.2%*
$[Ca^{2+}]$ 1 mmol/L (−)	53.8% ± 9.1%	27.6% ± 0.6%

Values represent means ± SD calculated from 6 independent experiments.

*Significant differences of cell viability with Ca^{2+} compared with cell viability without Ca^{2+} ($p < .05$).

After exposure to 500 μg/mL CDDP with 1 mmol/L Ca^{2+} for 2 minutes, the cells in the multiplates were cultured for 12 hours. Then, we performed the MTT assay in the same manner as chemosensitivity testing. The OD of the KB cells exposed to CDDP was 0.44 ± 0.08, whereas the OD of the KBrc cells was 0.53 ± 0.05. The viability of the KB cells was decreased compared with that of the KBrc cells. No significant difference of control OD was observed between the cell lines (Table 8).

DISCUSSION

In order to elucidate the mechanisms of CDDP resistance, we performed chemosensitivity tests and measurement of the intracellular Pt concentrations as well as the $[Ca^{2+}]$i changes in KB and KBrc cells. The KBrc cells were resistant to CDDP and to CDDP

TABLE 7. CELL VIABILITIES OF KB CELLS AND KBRC CELLS 6 AND 12 HOURS FOLLOWING EXPOSURE TO 500 μG/ML CDDP WITH CIRCULATION PUMP (TRYPAN BLUE DYE EXCLUSION)

	6 h	*12 h*
KB cells	36.2% ± 5.4%*	14.9% ± 2.4%*
KBrc cells	61.9% ± 4.8%	20.7% ± 0.5%

Values represent means ± SD calculated from 6 independent experiments.

*Significant differences of cell viabilities of KB cells in comparison with KBrc cells ($p < .05$).

TABLE 8. OPTICAL DENSITIES OF KB CELLS AND KRBC CELLS 12 HOURS AFTER EXPOSURE TO 500 µG/ML CDDP FOR 2 MINUTES (MTT ASSAY)

	OD in Control	*OD in Exposure to CDDP*
KB cells	0.66 ± 0.05	0.44 ± 0.08*
KBrc cells	0.68 ± 0.03	0.53 ± 0.05

Values represent means ± SD calculated from 8 independent experiments.

MTT — 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyl-2H tetrazolium bromide, OD — optical density.

*Cell viability of KB cells was lower than that in KBrc cells ($p < .05$).

analog drugs that were structurally related to CDDP. Against 3 kinds of anthracycline drugs, KBrc cells were not resistant compared with the KB cells. After exposure to CDDP or CBDCA, the intracellular Pt concentrations in KBrc cells were lower than those in KB cells. Though these results did not reflect a reduced level of drug binding to DNA of the tumor cells, decrease of intracellular Pt accumulation may be one of the possible mechanisms responsible for resistance of KBrc cells. These differences of intracellular Pt accumulations involved the influx and/or efflux of drugs, which are related to cell membrane function. Andrews et al[23] reported that intracellular CDDP accumulation was inhibited by ouabain, a specific inhibitor of Na^+-K^+ ATPase, and that either CDDP accumulation was dependent on the electrical gradient across the plasma membrane, or CDDP was transported by the Na^+-K^+ ATPase itself. CDDP-resistant L-1210 cells were shown to have altered calcium channels as compared with their parental L-1210 cells.[24] Our results suggest that there was a functional difference in the cell membrane, especially the signal transduction pathway, between the KB and the KBrc cells.

In order to determine the mechanisms underlying the function of the cell membrane exposed to CDDP, we measured the $[Ca^{2+}]i$ changes in both cell lines during exposure to CDDP. In general, $[Ca^{2+}]i$ is one of the most important ions in the relationship of cellular metabolism, signal transduction,[9] and disturbances in calcium homeostasis that can cause cell damage and cell death.[10] Burkey et al[10] reported that the intracellular calcium changes in tumor cells were associated with the in vitro cytotoxicity of lymphocyte activated killer cells and natural killer cells. In that report, they suggested that a high $[Ca^{2+}]i$ may kill tumor cells by direct osmotic effects, by poisoning of the cell's essential components, or by starting a cascade of enzymatic activities via a second messenger system.[10] That the cytotoxicity of cytokines such as tumor necrosis factor and interferon-γ may be mediated by an elevation of the $[Ca^{2+}]i$ in tumor cells has also been reported. Belloma et al[11] reported that tumor necrosis factor induced apoptosis by an increase of $[Ca^{2+}]i$ accumulation. Klein et al[12] also reported that an increase in both $[Ca^{2+}]i$ and protein kinase C activity was required for a functional response to interferon-γ. SRI 62-834, an antineoplastic ether lipid, brought about a concentration-dependent, biphasic rise in intracellular calcium.[25] In that report, Lazenby et al[25] stated that the nature of the relationship between the calcium rise and the cytotoxicity observed in the two cell lines will depend not only on the size of the rise observed, but also on the inherent sensitivity of each cell line to sustained elevations of intracellular calcium, determined by such criteria as the activity of calcium efflux pumps and/or intracellular mechanisms of calcium sequestration. CDDP is also known to have some influence on the cell membrane besides its well-known action on the nucleus. That the affinity and the number of epidermal growth factor receptors located in the cell membrane can be altered by CDDP has been reported.[26,27] Epidermal growth factor induced a receptor-mediated hydrolysis of inositol phospholipids for signal transduction pathways.[28,29] Bergler et al[30] reported that the possible site of action of CDDP and 12-0-tetradecanoylphorbol-13-acetate was considered to be the epidermal growth factor receptor and its signal transduction.

From our experiments, a rapid elevation of the $[Ca^{2+}]i$ in KB cells was observed after exposure to CDDP in a dose-dependent manner. Further, the calcium channel blocker LaCl3 inhibited the $[Ca^{2+}]i$ increase during exposure to CDDP. This result indicates that the $[Ca^{2+}]i$ increase induced by CDDP is not simply a result of membrane leakage, but is also mediated through a special calcium channel or receptor of CDDP on the cell membrane. CDDP caused an increase of $[Ca^{2+}]i$ even in the absence of extracellular Ca^{2+}. Therefore, since not all of the cells tested in the present study increased their $[Ca^{2+}]i$, this result indicates the possibility of Ca^{2+} release from internal stores. Though we cannot preclude the possibility that a small amount of Ca^{2+} influx from the extracellular space may have induced Ca^{2+} release from internal stores, this small amount of $[Ca^{2+}]i$ increase may bypass the signal transduction pathways such as inositol and/or protein kinase C pathways. Additional data are required to fully elucidate the role of Ca^{2+} release from internal stores during exposure to CDDP.

In the measurements of the cell viabilities of KB cells exposed to CDDP with and without Ca^{2+}, the cell viability with Ca^{2+} was lower than that without Ca^{2+}. The rises of $[Ca^{2+}]i$ induced by CDDP might effect cell damage besides DNA impairment.

When we compared the [Ca^{2+}]i changes on exposure to high-dose CDDP between KB and KBrc cells, a slower recovery and higher sustained elevation of [Ca^{2+}]i were observed in KB cells. Moreover, cell viabilities of KB cells exposed to high-dose CDDP were lower than those of KBrc cells. These results lead to a hypothesis in which KBrc cells possess molecular mechanisms in their cell membranes that enable them to minimize [Ca^{2+}]i changes in the presence of CDDP. Since little is known about which types of calcium channels are involved in the CDDP-induced elevation of [Ca^{2+}]i and the CDDP-resistant mechanism, we are now performing further experiments.

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REVISION SURGERY OF A STENOTIC SPEAKING SHUNT IN NEAR-TOTAL LARYNGECTOMY PATIENTS

CHIH-YING SU, MD

KAOHSIUNG, TAIWAN

A near-total operation to create a speaking shunt is generally considered a suitable option in most T3 laryngopharyngeal cancer cases. However, some of these cases incur postoperative problems, including aspiration and shunt stenosis. In this 8-year study, 15 of 60 patients who had received near-total laryngectomies could not speak well with their shunts. Revision surgery was performed on 11. Under local anesthesia, the lumen of the stenotic segment of the shunt was opened. A 4-F silicone tube was stented in the shunt. Then, for mildly stenotic cases, the small mucosal defect of the shunt was covered by a sternohyoid myofascial flap. For moderate to severe cases, the larger mucosal defect was reconstructed with a free skin graft. Thereafter, a sternohyoid muscle flap was used to cover the skin graft, which would finally form the inner lining of the new speaking shunt. The outcome was that 9 of the 11 patients acquired intelligible shunt speech. These results demonstrate that the augmentation procedures described herein have considerable potential for restoring the function of a stenotic speaking shunt in a near-total laryngectomee.

KEY WORDS — hypopharynx, larynx, near-total laryngectomy, neoplasm, revision, speaking shunt stenosis.

INTRODUCTION

Near-total laryngectomy or near-total laryngopharyngectomy is an effective treatment option in most T3 and some T4 laryngopharyngeal cancer patients.[1-5] Except for a small, cancer-free strip of laryngeal mucosa connecting the pharynx and trachea over a normal arytenoid, this surgical procedure involves a near-total resection of the laryngopharynx and the creation of a composite speaking shunt that serves as a sphincteric tracheopharyngeal conduit. This voice preservation procedure, which was first described by Pearson et al,[1-3] is believed to be an oncologically safe procedure for the ablation of laryngopharyngeal cancers. It has since been successfully practiced by several physicians.[6-9]

The valved speaking shunt (Fig 1) in a near-total laryngectomee controls the volume of airflow into the pharynx during speech and contracts to prevent aspiration when swallowing. Generally speaking, the same complications associated with total laryngectomies have been encountered after near-total laryngectomies, but with the latter, aspiration and shunt stenosis can also be complications. Of 41 patients in the Pearson's 1986 series,[3] there were 2 with "high-pressure" voice and 3 with true shunt stenosis. DeSanto et al[5] reported that 4 of their 28 supraglottic

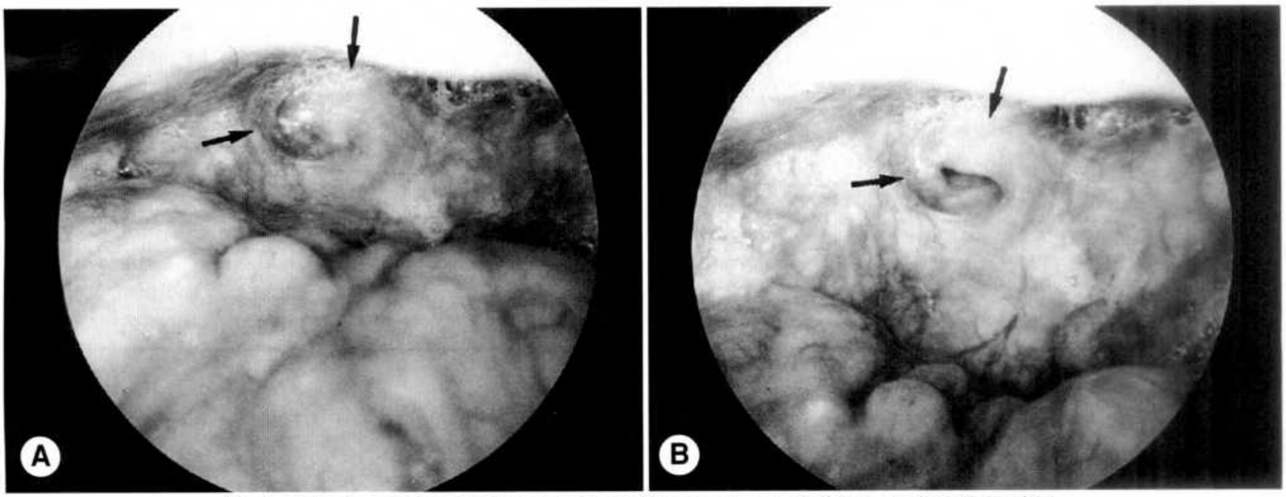

Fig 1. Valved speaking shunt (arrows). A) Closed, at rest. B) Open, during phonation.

From the Department of Otolaryngology, Chang Gung Medical College and Chang Gung Memorial Hospital, Kaohsiung, Taiwan. Supported in part by the National Science Council, Republic of China (NSC87-2314-B182-004) and the Chang Gung Medical College (CMRP 569).

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CORRESPONDENCE — Chih-Ying Su, MD, Dept of Otolaryngology, Chang Gung Memorial Hospital, 123 Ta Pei Road, Niao Sung Hsiang, Kaohsiung Hsien, Taiwan.

OUTCOME OF REVISION SURGERY OF THE STENOTIC SPEAKING SHUNT IN 11 NEAR-TOTAL LARYNGECTOMEES

							Shunt Speech	
Patient	*Age*	*Sex*	*Primary Cancer*	*Previous RT*	*Augmentation Procedure*	*Complications*	*Pre-revision*	*Post-revision*
1	73	M	Glottis	–	SMF*	Dislodgement of stent	No	Fair
2	62	M	Pyriform sinus	–	SMF + SG*	Dislodgement of stent	Poor	Excellent
3	72	M	Glottis	+	SMF + SG	Infection	No	No
4	71	M	Glottis	+	SMF	Infection	No	No
5	57	M	Pyriform sinus	–	SMF		No	Excellent
6	62	M	Supraglottis	–	SMF		Poor	Good
7	51	F	Pyriform sinus	–	SMF + SG		No	Good
8	58	M	Pyriform sinus	+	SMF	Infection	Poor	Fair
9	62	M	Hypopharynx	–	SMF		No	Good
10	57	M	Glottis	+	SMF + SG	Mild infection	No	Good
11	48	M	Pyriform sinus	+	SMF + SG*	Graft necrosis	Poor	Good

RT — radiotherapy, SMF — sternohyoid muscle flap, SG — skin graft.

*Three patients received procedures twice. Patient 11 was successfully salvaged with sternocleidomastoid myoperiosteal flap.

cancer patients who received a near-total operation could not speak with their shunts. Bouginage of the shunt followed by immediate use of stenting has been recommended for stenosis.[3] In this report, the author presents an open technique for revision of the stenotic speaking shunt.

MATERIALS AND METHODS

From January 1988 to February 1996, near-total operations with the creation of a composite speaking shunt were carried out on 60 laryngopharyngeal cancer patients. This series included 5 stage T2, 30 stage T3, and 25 stage T4 cases. Of the 60 patients, 45 (75%) were successfully rehabilitated with intelligible shunt speech. Fifteen operations (25%) resulted in a high-pressure voice or an inability to speak. Two of these 15 complications were due to local tumor recurrence at the shunt; 13 patients suffered from true stenosis of the shunt. In 11 of the 13 patients, revision surgery was performed to enlarge the lumen of the stenotic shunt (see Table). The 2 shunt recurrence cases were salvaged with completion laryngectomies.

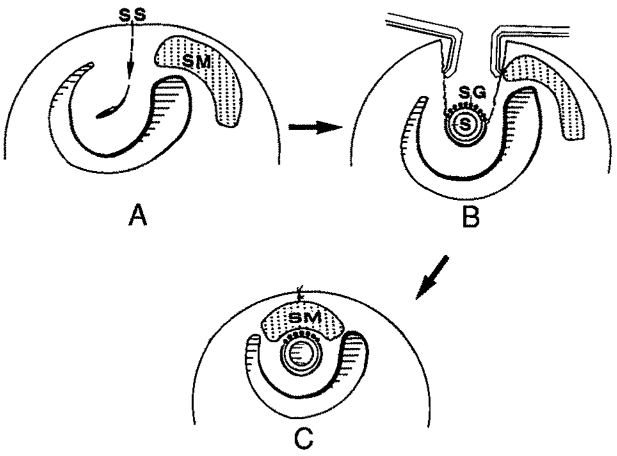

Fig 2. Diagram demonstrating augmentation procedures. A) Transverse section of neck with stenotic speaking shunt (SS) and sternohyoid muscle (SM). B) Shunt lumen is opened and stented with silicone (S). Mucosal defect of shunt is repaired with skin graft (SG). C) Skin graft is then covered by sternohyoid muscle flap (SM).

Surgical Technique. The augmentation procedures (Fig 2) were carried out under local anesthesia. First, the throat and the tracheostoma of the patient were sprayed with 8% lidocaine hydrochloride. The anterior neck was locally infiltrated with 2% lidocaine hydrochloride containing 1:80,000 epinephrine. A 3-cm vertical incision line was made starting approximately 0.5 cm above the tracheostoma (Fig 3A). After lysis of the surrounding scar and fibrotic tissue, the lumen of the stenotic segment of the shunt was thoroughly opened. A 4-F silicone tube was used as a stent. It could be introduced upward via the shunt, and found in the oropharynx. With the aid of a transnasal rubber catheter, the top end of the silicone tube could be drawn up into the nostril and firmly fixed at the nasal septum with 3-0 nylon sutures. Meanwhile, the bottom segment of the tube was located just within the speaking shunt to serve as a stent (Figs 2 and 3A). For cases of mild stenosis, the mucosal defect of the shunt was covered with a sternohyoid myofascial rotation flap. For moderate and severe cases, the large mucosal defect was repaired with a free skin graft (Figs 2 and 3B). A sternohyoid muscle flap was used to cover the skin graft, which would finally form the inner lining of the new speaking shunt. At the end of the procedure, a Penrose drain was set and a light pressure dressing was applied over the anterior neck. The patient then was prescribed nasogastric tube feeding for at least 5 days.

The silicone stent was removed 3 to 4 weeks later

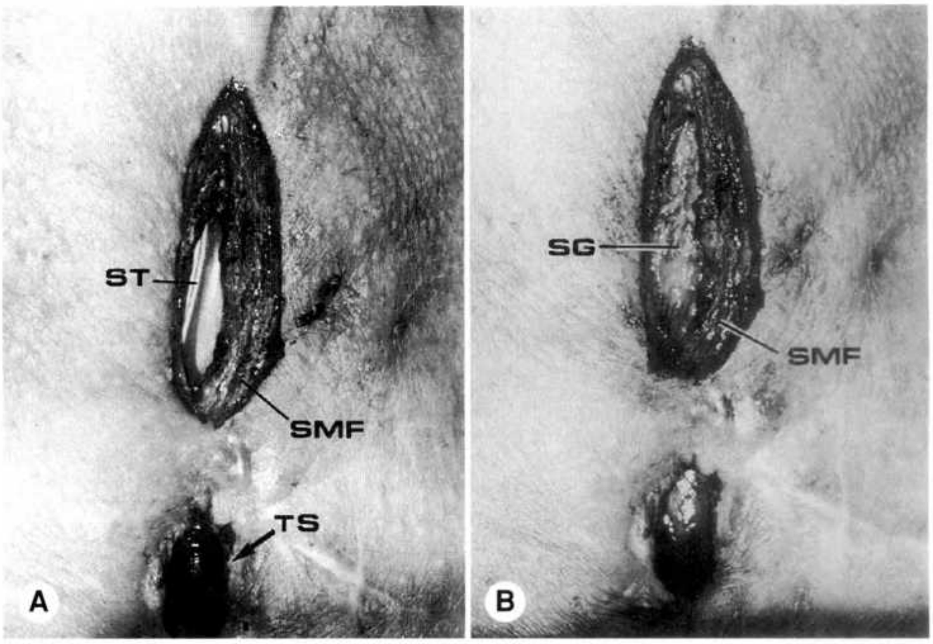

Fig 3. Intraoperative photographs illustrating A) vertical incision line above tracheostoma (TS). Lumen of stenotic speaking shunt is opened and stented with silicone tube (ST). B) Mucosal defect of shunt is repaired with skin graft (SG), which is then covered by sternohyoid muscle flap (SMF).

in the outpatient clinic. The patient was encouraged to occlude the tracheostoma and begin vocal rehabilitation. After the completion of speech therapy, the quality of speech was evaluated by both the surgeon and the speech pathologist. For reporting purposes, results were rated as follows. Fluent, intelligible speech of nearly normal quality was defined as "excellent"; husky, fluent, and intelligible speech was defined as "good"; tight, husky speech that interfered with intelligibility was rated as "fair"; and a high-pressure voice or an inability to speak was called "poor."

RESULTS

Eleven patients who had stenotic speaking shunts received the augmentative surgical procedures (see Table). These 11 represent 18% of our near-total series. Three of the patients received two procedures because of premature prolapse of the stent or necrosis of the graft after the first procedure. Eighty-two percent (9/11) of the patients acquired intelligible shunt speech. In 2 unsuccessful cases, the causes of failure were wound infection and graft necrosis. Both were patients who had been treated with previous irradiation.

In this series, 3-year follow-up results are available for the first 38 of the 60 patients who received near-total laryngectomies or near-total laryngopharyngectomies. The 3-year local recurrence rate was 10.5% (4/38). The overall survival was 60.5% (23/38).

DISCUSSION

The near-total operation to create a composite speaking shunt is an appropriate primary treatment in T3 and some T4 cancers of the larynx and pyriform sinus. Patients whose hemilarynx, pyriform sinus of the contralateral side, and interarytenoid area are still free of tumor invasion are candidates.[1-5,8] Although near-total surgery does not preserve nasal breathing, it does preserve the patient's voice.

In forming an adequate speaking shunt, Pearson[3] recommended a shunt lumen of 6 mm in diameter. In many laryngeal cancers that fix one vocal cord, creation of an adequate shunt requires that a pharyngeal flap be folded down to augment the diameter of the shunt. If the shunt is formed or heals too tightly, the patient speaks with a high-pressure voice. If severe stenosis of the shunt develops, the patient may be unable to pass air through the shunt and be unable to speak.[3] In my experience, poor shunt speech can be due to an inadequate shunt lumen or to compression by the fibrotic band and scar tissue surrounding it. The open augmentation procedure described here was designed to lyse fibrotic tissue around the shunt and to enlarge the shunt lumen with stenting and grafting.

Under local anesthesia, the patient usually does well, without much discomfort. The physician can manipulate the shunt more easily without an intubation tube in the tracheostoma. During the procedure, the whole stenotic segment of the shunt must be thoroughly opened until a 4-F silicone tube can be smoothly introduced into the oropharynx via the shunt. For a mildly stenotic shunt with a small mucosal defect after reclosure over a stent, epithelialization along the fascia plane of the overlying sternohyoid muscle may easily occur. The mucosal defect does not need skin grafting. For a more severely stenotic shunt, the large mucosal defect is better repaired with a free skin graft followed by coverage with a strap muscle flap on the skin graft (Figs 2 and 3). For a postirradiated or diabetic patient, wound infection is a prominent postoperative problem (see Table). Graft necrosis and restenosis of the speaking shunt may occur if the infection is not well controlled. The 2 unsuccessful cases in this series had received a full dose of radiotherapy before the augmentation surgery was performed. All the patients who had had no previous irradiation responded well to this surgical procedure.

Three patients received procedures twice, because of either a premature dislodgement of the stent or a wound infection. If premature dislodgement of the silicone tube occurs and the duration of stenting is less than 2 weeks, shunt speech usually fails to develop. If the silicone tube stent (which is hollow) is secured with its top end in the pharyngeal lumen, aspiration would be a problem. For these reasons, it is suggested that the top end of the silicone tube be firmly fixed at the nasal septum with doubled 3-0 nylon sutures. The bottom segment of the tube should remain in the speaking shunt for at least 3 to 4 weeks. Adequate passive drainage, parenteral antibiotics, and nasogastric tube feeding for several days are important for the prevention of wound infection.

In conclusion, revision augmentation procedures have considerable potential for restoring voice in near-total laryngectomy patients with shunt stenosis.

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IMAGING CASE STUDY OF THE MONTH

CHRONIC MAXILLARY ATELECTASIS IN A CHILD

ERIK S. KASS, MD
BETHESDA, MARYLAND

SALAH SALMAN, MD
BOSTON, MASSACHUSETTS

WILLIAM W. MONTGOMERY, MD
BOSTON, MASSACHUSETTS

INTRODUCTION

Chronic maxillary atelectasis (CMA) is defined as a persistent decrease in the sinus volume of the maxilla due to inward bowing of one or more antral walls.[1] Symptoms are usually present, and nasal endoscopy shows fontanelle retraction. The diagnosis of this uncommon condition can be made from the characteristic computed tomographic (CT) findings of sinus opacification and lateral bowing of the medial infundibular wall (MIW).[1] These findings are thought to result from valvular occlusion of the natural ostium by a flaccid MIW that acts like a flap.[1,2] Ostial occlusion leads to the development of negative sinus pressure, and this pressure change is thought to be analogous to that which occurs with eustachian tube dysfunction.[2,3] Chronic maxillary atelectasis is confirmed by the presence of negative sinus pressure on manometric measurement.[2]

Although CMA is suspected to occur in the pediatric population,[4] a confirmed case in this age group has not been previously reported in the literature. We present a child with manometrically confirmed CMA and review the characteristic radiographic findings. Consideration is given to the association of this condition with maxillary sinus hypoplasia.[5]

CLINICOPATHOLOGIC FINDINGS

A 9-year-old boy was referred by his pediatrician for recurrent sinusitis. He complained of intermittent dull facial pain over the right cheek that worsened during upper respiratory tract infections, and his mother reported frequent episodes of halitosis. There was no past history of facial trauma or surgery. Physical examination revealed an enlarged middle meatal space on the right due to a laterally retracted MIW. The remainder of the head and neck examination was unremarkable. A CT scan showing complete right maxillary sinus opacification with fontanelle retraction was unchanged from a scan done 21 months earlier.

A right endoscopic middle meatal antrostomy was performed. Intraoperative findings included complete occlusion of the right maxillary sinus ostium by the MIW. The posterior aspect of the retracted MIW was flaccid, and it appeared to lack an osseous attachment to the inferior turbinate. The MIW was also found to possess prominent height posteriorly. Static pressure measurements were taken of the maxillary sinuses with an 18-gauge needle probe according to a previously described technique.[2] A pressure of −8 cm H2O was found within the right antrum, while isobaric pressure was found in the left antrum (see Table). The pressure rose to zero when the right MIW was peeled away from the lateral infundibular wall with an ostium seeker. A middle meatal antrostomy was created, and the antral mucosa was minimally thickened following evacuation of thick and tenacious secretions. The histopathology showed mild chronic inflammatory changes of the mucosa.

MAXILLARY SINUS PRESSURE, VOLUME, AND SURFACE AREA MEASUREMENTS TAKEN 21 MONTHS APART

	Pressure (cm H2O)	*Volume (mL)*		*Surface Area (cm^2)*	
Age (y)	*Right*	*Right*	*Left*	*Right*	*Left*
6.8		2.25 ± 0.004	6.42 ± 0.005	10.49 ± 0.046	17.19 ± 0.030
8.5	−8	2.32 ± 0.015	7.54 ± 0.017	11.37 ± 0.028	19.56 ± 0.132

Volume and area measurements are mean ± SD.

From the Department of Otology and Laryngology, Harvard Medical School and the Massachusetts Eye and Ear Infirmary, Boston, Massachusetts.

CORRESPONDENCE — Erik S. Kass, MD, Laboratory of Tumor Immunology and Biology, National Cancer Institute, Bldg 10, Room 8B07, 10 Center Dr MSC 1750, Bethesda, MD 20892-1750.

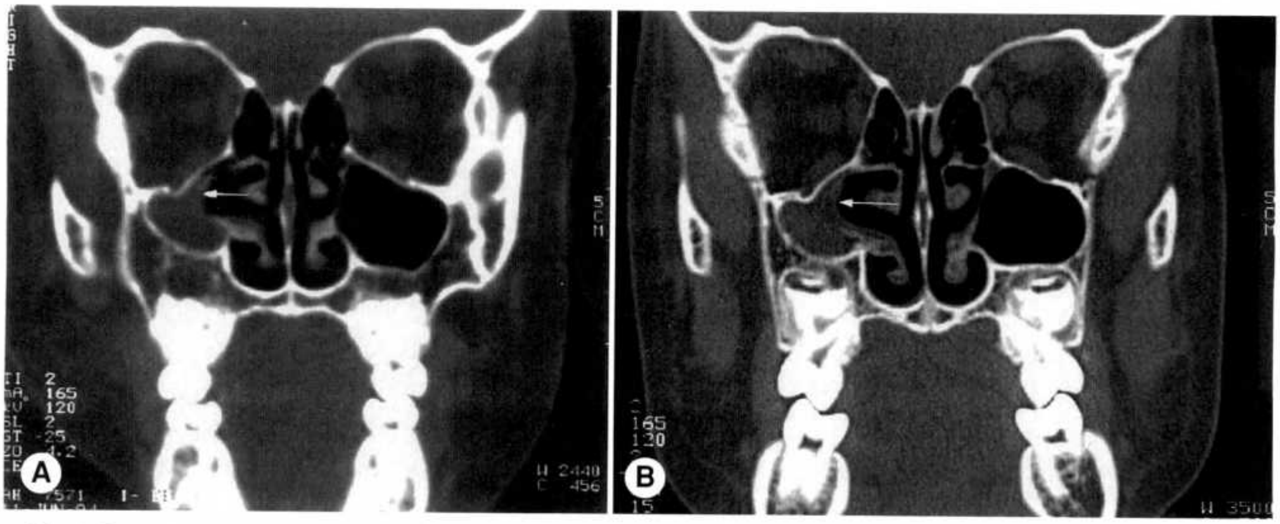

Fig 1. Coronal computed tomography scans. A) Complete opacification of right maxillary sinus and smooth concavity to medial aspect of fontanelle (arrow) consistent with chronic maxillary atelectasis. Right antrum appears to be considerably smaller than left antrum. B) Scan obtained 21 months later, again showing complete opacification of right maxillary sinus and smooth concavity to medial aspect of fontanelle (arrow) and virtually no change in size. Left antrum appears to have increased in size.

The patient did well postoperatively and was symptom-free with a patent middle meatal antrostomy at his 6-month follow-up visit.

RADIOLOGIC FINDINGS

A coronal CT scan showed complete opacification of the right maxillary sinus with lateral retraction of the right MIW and fontanelle. The right antrum was smaller than the left, and had a greater amount of intramedullary bone surrounding it, consistent with incomplete pneumatization. Comparison of the coronal CT scan with one obtained 21 months earlier showed no apparent change in the size of the right antrum, while enlargement was seen in the left maxillary sinus (Fig 1).

Antral volume and surface area measurements were taken from coronal CT sections (see Table and Fig 2). The scans were traced and registration (reference) points were digitized manually with an electronic data tablet. Standard computerized planimetry equations were used to determine the area of each region in each section. The volumes were then calculated as the sum of the 2- and 5-mm-thick sections with areas equal to the region areas. This "stacked coin" volume approximation is accurate within 10% of the actual volume. The normal-appearing left sinus had a volume of 6.42 mL, and this increased by 1.12 mL over a 21-month period, while the volume within the atelectactic right antrum was 2.25 mL (35% of normal sinus volume) and showed virtually no change over this same time interval.

DISCUSSION

Predisposition to the development of CMA is

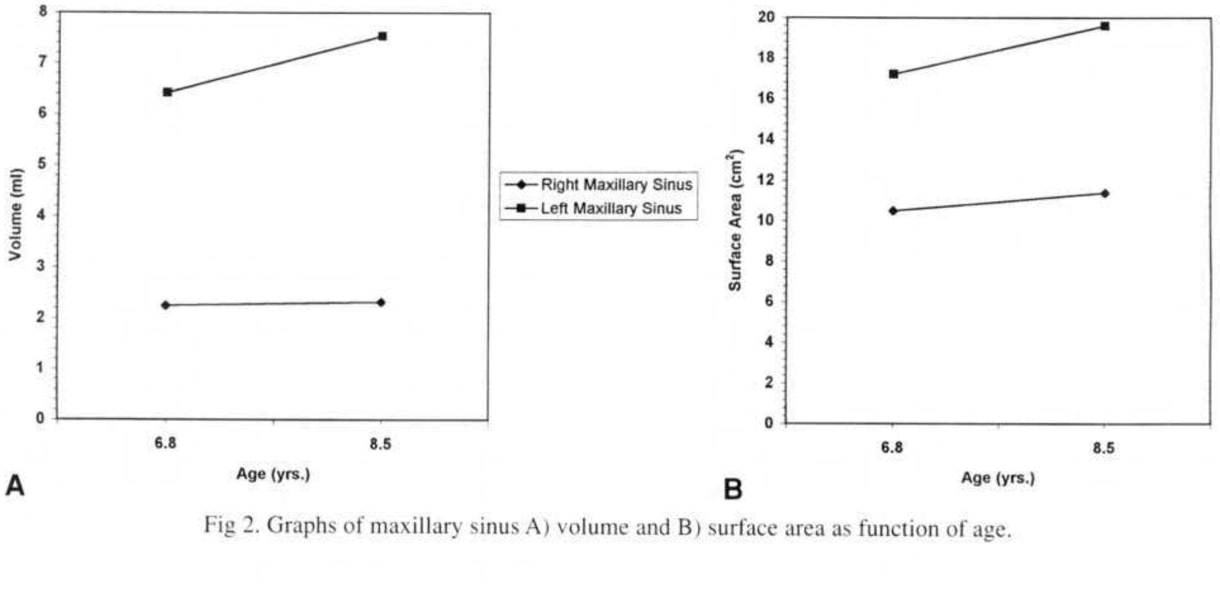

Fig 2. Graphs of maxillary sinus A) volume and B) surface area as function of age.

thought to be due to a deficient posterior attachment of the uncinate process to the inferior turbinate bone.[1,2] Prominent vertical length of the posterior aspect of the infundibular wall and a narrow infundibular passage are also considered important contributors.[1,2] The cause of the deficient uncinate process may be congenital in children with CMA.[1,2]

Middle meatal inflammation is postulated to lead to flap valve occlusion of the natural ostium.[2] Gas resorption takes place in the totally occluded sinus, and negative pressure ensues.[2] Eventually, persistent negative static pressure leads to the accumulation of fluid within the enclosed space.[2] This process is self-perpetuating and analogous to the events that occur in patients with middle ear effusions due to eustachian tube dysfunction.[1-3]

Flap valvular occlusion of the natural ostium is a passive and reversible process and is based fundamentally on the presence of negative pressure within the sinus.[2] If pressure within the sinus becomes positive transiently, spontaneous drainage and the collection of air pockets could occur.[2] The diagnosis of CMA may become more difficult to make in this instance.

This case demonstrates the characteristic radiographic features found in pediatric CMA. The chronically retracted appearance of the MIW and fontanelle, along with antral opacification, were diagnostic of the condition. The right maxillary sinus also showed hypoplastic features that were confirmed by comparison with volume and surface area measurements of the opposite side.

Two etiologic factors that are thought to lead to sinus underdevelopment are chronic inflammation and ostial obstruction.[5,6] Since both of these factors occur in CMA, some degree of hypoplasia would be expected in pediatric patients having the disorder for any appreciable amount of time. An association between CMA and hypoplasia is already known to exist in adults.[1] The overall impact of CMA on sinus underdevelopment is uncertain, since the prevalence of CMA-associated maxillary sinus hypoplasia in the pediatric population is not known. Maxillary sinus hypoplasia per se is not generally thought to cause morbidity, although vertical dystopia has been reported in children with this condition.[7]

In the case presented, no significant developmental change in right maxillary sinus volume or surface area occurred over a 21-month interval. These findings were consistent with the results of a study by Proctor and Naclerio.[8] Whether, and to what extent, relief of ostial occlusion would lead to resumption of pneumatization in a hypoplastic sinus is unclear and requires further study. At this stage in our understanding, a middle meatal antrostomy is recommended for children with CMA who have symptoms attributable to sinus blockage.

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PATHOLOGY CONSULTATION
MUCOSAL MELANOMAS OF THE HEAD AND NECK

JOHN G. BATSAKIS, MD
PATRICIA SUAREZ, DDS
ADEL K. EL-NAGGAR, MD, PHD
HOUSTON, TEXAS

Mucosal melanomas of the head and neck, as a group, are rapidly lethal neoplasms with site-specific differences in survival. They are preponderantly thick melanomas and as a consequence can present with cervical lymph node metastasis with or without distant spread. Long-term survival is unusual to rare; 5-year survival is poor, and 10-year survival, dismal. Whether this life-consuming nature is due to a biologic characteristic or related to late discovery is unknown. Because of the accelerated course and obvious differences in epidemiology, mucosal melanomas should be considered separate from melanomas of the skin.

Noncutaneous, nonocular melanomas are greatly outnumbered by their cutaneous counterparts. Table 1[1,2] presents data from 2 studies, each relatively free of a selective referral bias, to underscore the uncommon occurrence of these types of melanoma. Most noncutaneous, nonocular melanomas are anorectal or are found on the female genitalia. Melanomas of head and neck mucosa are far less common.

INCIDENCE OF MUCOSAL MELANOMAS OF HEAD AND NECK

Mucosal melanomas of the upper aerodigestive tracts are notably less frequent than those arising in the anogenital mucosa and hence present special problems in estimating their incidence. Institutional referral patterns and putative geographic differences contribute to the difficulty. Chiu and Weinstock,[2] authors of a report founded on 9 population-based cancer registries, indicate that 182 (25%) of 740 noncutaneous, nonocular cases were oronasal in origin, and these represented 0.42% of all melanomas registered during a 19-year period. The overall incidence of oronasal melanomas was $0.041/10^5$ per year. Incidence rates for oral and sinonasal melanomas were $0.012/10^5$ per year and $0.018/10^5$ per year, respectively.

TABLE 1. FREQUENCY OF MELANOMA INCLUDING CUTANEOUS AND NONCUTANEOUS CASES

	American College of Surgeons Survey[1]	*Nine Population-Based Cancer Registries*[2]
Total cases of melanoma	10,564	43,304
Cutaneous	9,887 (93.6%)	40,320 (93.1%)
Ocular	520 (4.9%)	2,449 (5.2%)
Noncutaneous, nonocular	157 (1.5%)	740 (1.7%)

A review of the literature in 1982 found 531 cases of head and neck mucosal melanomas.[3] Manolidis and Donald[4] added 420 cases in 1997, bringing the total to nearly 1,000 cases. In terms of proportion of all melanomas, primary mucosal melanomas of the head and neck represent 0.4% to 1.8% of the total.[4] In Finland, the ratio of head and neck mucosal melanomas to all malignancies is estimated to be 1 to 11,626, and the ratio of the mucosal melanomas to all head and neck melanomas to be 1 to 105.[5] Others report a range of 6% to 20% mucosal melanomas in cohorts of all melanomas of the head and neck.[3]

There are differences of opinion as to whether the oral cavity or the sinonasal cavity is the most afflicted site. Here again, institutional reporting bias is at work. It is likely there is no significant difference. Uncontested is the relative rarity of primary melanomas of the larynx. Tables 2 and 3, prepared from data presented by Manolidis and Donald,[4] give the reported sites of origin for oral and sinonasal mucosal melanoma.

Because the number of cases is small, investigators often lump separate anatomic sites into broad categories such as upper aerodigestive tract or oronasal. If there are biologic differences related to anatomic location, they can be obscured by this type of reporting.

CLINICOPATHOLOGIC FEATURES

There is a persistent thesis that mucosal melano-

From the Department of Pathology, University of Texas M. D. Anderson Cancer Center (Batsakis, El-Naggar), and the Department of Oral Pathology, University of Texas Dental Branch (Suarez), Houston, Texas.

CORRESPONDENCE — John G. Batsakis, MD, 1701 Hermann Dr, #3304, Houston, TX 77004.

TABLE 2. ORAL CAVITY MELANOMA (N = 430)

Oral Site	*No. of Cases*		
Palate			
NOS	154	44.0%	77.2%
Hard	31		
Soft	4		
Alveolus			
Maxillary	90	33.3%	
Mandibular	36		
Alveolus NOS	17		
Buccal mucosa	33		
Lip	30		
Tongue	10		
Floor of mouth	4		
Oral cavity NOS	21		

Modified from Manolidis and Donald.[4]

NOS — not otherwise specified.

TABLE 3. SINONASAL MELANOMA (N = 346)

	No. of Cases	
Nasal site		
Nasal NOS	159	283 (81.8%)
Septum	44	
Lateral wall	44	
Inferior turbinate	12	
Middle turbinate	7	
Turbinate NOS	12	
Floor of nose	5	
Sinus site		
Maxillary	32	63 (18.2%)
Ethmoid	8	
Frontal	2	
Sinus NOS	21	

Modified from Manolidis and Donald.[4]

mas of the upper respiratory tracts are sufficiently biologically different to warrant a separate consideration from their far more numerous cutaneous counterparts.[3,6,7] To explore this possibility, we have undertaken a review.

Common to all melanomas is origin from melanocytes in the epithelium or subepithelial tissues and a worsening of outlook with an increasing depth and/or thickness of the primary lesion. For cutaneous and mucosal melanomas, the single most powerful predictor of survival is said to be the presence or absence of regional lymph node metastasis.[8,9] For melanomas of the skin, it has been estimated that lymph node metastases diminish the 5-year survival by approximately 40%.[10,11] Further, it is the *number* of involved lymph nodes rather than the size of an affected node that is more important. Once spread to regional lymph node(s) has occurred, prognostic factors derived from the study of the primary melanoma add very little in comparison to the number of involved lymph nodes.

Tumor Thickness. Direct comparisons between melanomas of the skin and melanomas of the upper aerodigestive tracts are difficult to make and consequently few in number. One example is that by Umeda and Shimada[12] (Table 4). Their data indicate that 1) the preponderance of cutaneous melanomas are stage I (localized), while about half of oral melanomas show regional lymph node metastasis, and 2) 57% of stage I cutaneous melanomas have a thickness of less than 1.5 mm, while 70% of stage I oral melanomas have a tumor thickness of greater than 4.0 mm.

Most oral melanomas are clearly more advanced at the time of diagnosis. The thicker the mucosal melanoma, the more likely regional lymph nodes will be involved, even if this morbid event is not manifest at diagnosis. The most common sites of nodal involvement are the submandibular and upper jugular lymph nodes (same as for squamous cell carcinoma of head cavity). The literature also suggests a propensity for bilateral lymph node metastasis.[12] Involvement of regional lymph nodes is also a forerunner of distant spread, although the latter is not necessarily preceded by the lymphatic spread.

Stage. Institutional reports concerning the status of cervical lymph nodes at the time of diagnosis for patients with head and neck mucosal melanoma indicate, albeit with some exceptions, that the melanoma is clinically localized (stage I) in up to 97% of patients.[13,14] This is in conflict with what Manolidis and Donald[4] relate in a review of 773 head and neck mucosal melanomas (oral cavity, pharynx, larynx, esophagus, and sinonasal tract). They found that approximately 19% of patients presented with regional lymph node metastases, and another 16% developed such metastases after the first course of treatment. Ten percent had stage III (distant spread) disease at first presentation.

Unfortunately, other studies dealing with large numbers of patients either do not offer information on stage at time of diagnosis or present such data in

TABLE 4. MELANOMA THICKNESS AND STAGE BY CUTANEOUS VERSUS ORAL SITES

	Cutaneous (%)		*Oral (%)*	
Melanoma Thickness (mm)	*Localized (Stage I; N = 2,151)*	*Nodal Metastasis (Stage II; N = 290)*	*Localized (Stage I; N = 10)*	*Nodal Metastasis (Stage II; N = 12)*
≤1.5	57	11	0	0
1.5 to 4.0	33	40	30	17
≥4.0	10	49	70	83

Modified from Umeda and Shimada.[12]

a combined (all sites) fashion. It is our presumption that of the 2 principal mucosal sites in the head and neck — oral cavity and sinonasal tract — melanomas of the nasal cavity and sinuses have a lower incidence of metastasis at discovery. This is supported by a further analysis of the data collected by Batsakis et al.[3] Cervical lymph node metastases were initially found in 12.8% of 172 sinonasal melanomas and in 28% of 111 oral melanomas. At first visit to a physician, 5 of 86 (5.8%) oral melanomas and 4 of 127 (3.1%) sinonasal melanomas had evidence of distant metastases.

Histopathologic Factors. Only tumor thickness, depth of invasion, and tumor volume have demonstrated significant correlation with regional metastasis and prognosis. Size of tumor has not, and other features such as neurotropism, microvascular invasion, histologic grade and typing, and angiogenesis have either shown no association or have had an inconsistent and non-independent relationship.

Preexisting "melanosis" continues to be studied. Except for racially related pigmentation, it has been nearly impossible to define in sinonasal melanomas. It is better judged in the oral cavity, but too often left undefined beyond the noncommittal "melanosis." Thirteen of 50 (26%) oral melanomas presented at the Western Society of Teachers of Oral Pathology Banff Workshop were said to have had preexisting pigmentation.[7] Others report that oral mucosal melanomas are preceded by oral melanosis in 30% to 37% of cases.[15] Of the nonracial oral pigmentations, the melanotic macule, a discrete area of increased basal layer pigmentation, is the most common.[3] Oral nevi are distant followers, and of these, intramucosal nevi are the most numerous; junctional nevi account for 3%, and blue nevi for 36%.[16] Nevi are most commonly found on the palate; macules, on the lower lip and alveolar gingiva.[16]

Grossly noticed pigmentation occurs in about 75% of oral melanomas but only in 50% of sinonasal melanomas.[3]

Oral and sinonasal mucosal melanomas arguably correspond to cutaneous acral lentiginous melanomas.[3,12] For oral cavity melanomas, Ohashi et al[6] have indicated that 77% of their cases showed a 2-phase growth pattern with radial and vertical growth phases. The radial phase was one of a lentiginous rather than a pagetoid pattern, similar to acral lentiginous melanoma. Twenty-three percent, however, had a vertical growth phase only. Of interest, most examples of the latter were amelanotic.

The oral pathologists at the Banff Workshop acknowledged the difficulty of applying the cutaneous melanoma classification to oral melanomas and, by allusion, to sinonasal melanomas.[7] They recommended the following, more simple classification: 1) in situ oral mucosal melanoma, 2) invasive oral mucosal melanoma, 3) invasive melanoma with an in situ component, and 4) atypical melanocytic proliferation (for equivocal lesions).

Survival. Survival has considerable variability. Manolidis and Donald[4] report the following: overall mean survival at 3 years was 39.2% (range 7% to 65%); at 5 years, mean survival was 17.1% (range 0% to 48%); and at 10 years, mean survival was 4.8% (range 0.5% to 26%). The after-10-year survival is dismal and has not exceeded 1.2%. Melanomas of the paranasal sinuses are the most life-consuming, with only rare 5-year survivors. The oral cavity melanoma is the next most lethal (5-year survival 12.3%). Intranasal melanomas show a 30.9% 5-year survival.

METASTASIS OF MELANOMA TO ORONASAL MUCOSA

For both oral and sinonasal melanomas, there is the admonition to exclude a metastatic melanoma. While the admonition is wise, possible confusion is less a problem for oronasal melanomas than it is for presumed primary melanomas of the larynx.[17,18] In a study of 8,823 patients with disseminated melanoma, metastases in the upper aerodigestive tracts were found in only 54 patients (incidence of 0.6%).[18] Sixty-two percent of the patients also had clinical evidence of disseminated disease at the time of discovery of the mucosal metastasis. Necropsy studies have found that 2% to 9.3% of patients with disseminated melanoma manifest head and neck mucosal metastases.[18] The metastases occur in a variety of upper aerodigestive sites and include the tonsils, tongue, nasopharynx, larynx, and lip. The tonsils are particularly susceptible. In the series of 8,823 patients, the palate and gingivae appear to have been spared. In the larynx, the supraglottis is most often involved.[18]

MELANOMA OF LARYNX

The incidence of a primary melanoma of the larynx is impossible to relate. Of all upper aerodigestive sites, the larynx most definitely requires exclusion of a metastasis.[19] This is not always possible, and that factor clouds data derived from the literature. Wenig[20] lists 46 antecedent cases prior to his presentation of 4 cases, but acknowledges the suspect nature of many of the reported examples. Reuter and Woodruff[21] have given similar disclaimers.

Demographically, primary melanomas of the larynx follow the findings of mucosal melanomas more cephalad in the aerodigestive tracts, eg, an almost exclusive white patient population and presenting most often in elderly men (more than 80%).[20] Most are supraglottic, with no documented example in the subglottis.[20]

Metastases to regional lymph nodes or to distant sites is observed in 80% of the patients; 27% for each is noted at the time of diagnosis.[21] Overall prognosis is very poor, with an average survival of less than 3.5 years. No more than 20% of patients live longer than 5 years after diagnosis.[20,21]

Laryngeal melanosis appears to be extremely rare. Gonzalez-Vela et al[22] added 4 examples to the 13 in the literature. Gross or immunohistochemically detected melanosis can affect any part of the larynx. In 9 (53%) of the 17 reported cases, the melanosis was associated with a coexisting laryngeal neoplasm (7 squamous cell carcinomas and 2 melanomas).

Smoking may play a role in a melanogenic metaplasia or increasing melanin proliferation by an activation of preexisting melanocytes.[22]

CONCLUSIONS

Rew's[23] description of the heterogeneity, biodiversity, and "bioperversity" of solid neoplasms is perhaps best exemplified by melanomas. Whether the mucosal melanomas of the head and neck show these features more than cutaneous melanomas was one of the questions this review was undertaken to answer. Are there enough biologic differences to separate the melanomas? Contrary to current thought, mucosal melanomas are not more often localized (stage I) than cutaneous melanomas. They are, however, more deeply invasive at diagnosis, again contradicting a stage I preponderance. Both of the 2 melanoma categories share the same significant histologic prognostic factors. A more rapidly lethal course is seen with the mucosal melanomas, and with that, earlier distant metastases. Whether this is due to failure to detect earlier or to an intrinsic feature of mucosal melanomas cannot be stated at this time. Certainly, there must be epidemiologic differences between the sun-afflicted cutaneous lesions and the solar-hidden mucosal melanomas. Preexisting "melanosis" is becoming more noted with the mucosal neoplasms. The premalignant nature of these lesions needs better definition.

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MENIERE'S DISEASE

The Fourth International Symposium on Meniere's Disease will be held in Paris, France, April 11-14, 1999. For information, contact Administrative and Scientific Secretariat, SOCFI/MENIERE '99, 14 rue Mandar, 75002 Paris, France; telephone 33 (0) 1 44 88 25 25; fax 33 (0) 1 40 26 04 44; e-mail fournier@socfi.fr.

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SAN FRANCISCO OTOLOGY, NEUROTOLOGY & SKULL BASE SURGERY UPDATE

The San Francisco Otology, Neurotology & Skull Base Surgery Update 1998 will be held November 5-7, 1998, in San Francisco, California. For information, contact University of California, Office of Continuing Medical Education, MCB-630, Box 0742, San Francisco, CA 94143-0742; telephone (415) 476-4251; fax (415) 476-0318; e-mail: inquire@ocme.ucsf.edu; web site: http://cme.ucsf.edu.

LETTER TO THE EDITOR

To the Editor:

Despite the prevalence of childhood otitis media,[1-3] the relevance of health costs, and wide acceptance that myringotomy with insertion of ventilation tubes (MVT) is a major treatment option,[4-9] there has been a paucity of reliable costing studies associated with otitis media management.[10] There are only two published costing studies, and these either offered a theoretical cost-effectiveness analysis[11] or a charge-based analysis.[12] Both studies were restricted to the United States and focused on otitis media with effusion in very young children. In this letter, we summarize the results of a Canadian cost analysis of MVT for children.

The cost analysis was performed from a health system perspective and was concerned with the identification and measurement of the expected cost of MVTs performed at a regional center for pediatric care ("study hospital"). A detailed treatment protocol was developed through a review of the literature and input from staff at the study hospital. The protocol, which focused exclusively on ambulatory surgical events, identified four treatment phases: preoperative assessment, surgical setup, surgical intervention, and postoperative recovery. For each phase, direct and indirect (or overhead) treatment costs for April 1, 1993, to March 31, 1994, were evaluated by means of standard procedures.[13]

The total cost of each uncomplicated MVT case (in 1994 Canadian dollars; $1.00 CDN equals approximately $0.75 US) was $472.63: physician service costs,[14] $202.82; other direct surgical costs, $86.23; preoperative assessment and recovery room costs, $67.22; and overhead costs, $116.36. Depending on the number of ventilation tubes inserted, costs per MVT case varied from a low of $421.34 to a high of $503.40 for a bilateral MVT.

The results outlined in this letter represent the first microlevel cost analysis of the health system resource costs associated with the provision of MVT surgery. Given the prevalence of childhood otitis media and the propensity to perform MVTs in North America, the total annual cost of MVT cases performed in North America is approximately $500 million (CDN), ie, 1 million cases at approximately $500 per case. Since these costs reflect only the health system costs incurred during a discrete ambulatory surgical event and are based on Canadian data, such estimates represent a conservative estimate of total MVT case costs.

Two US studies[11,12] reported MVT operative case charges that were between two and four times greater than our estimates, though the distribution of charges across the various components of care were similar. These charges ranged from a low of $826.73 for a Colorado Medicaid reimbursed MVT case in 1992[11] to a high of $1,617 for a 1992 Denver private practice[11] and $1,581.09 for all payor charges in 1990.[12] While a portion of these "cost" differences may be attributed to variations in the funding and delivery of health services between Canada and the United States, much of the difference may be due to the use of charges in the US studies and costs in our analysis. It is widely recognized that charges may bear little relationship to variations in resource costs,[15-17] as they may be modified for marketing or cross-subsidization reasons.

Our analysis was limited in three main respects. First, a partial health system perspective was taken instead of a more comprehensive societal perspective that also considers costs incurred prior to and following the discrete ambulatory surgical event. Second, the potential benefits or cost-savings attributable to MVT surgery were ignored. Third, MVT case costs were based on practices at the study hospital and, consequently, may be limited in their generalizability.

Given the prevalence of otologic disorders and the importance of costs, further studies using valid and reliable microlevel cost estimates are needed for alternative management options for patients. As such, the present analysis should be seen as one important step toward a full economic evaluation of treatment options for childhood otitis media.

PETER C. COYTE, MA, PHD
CARL V. ASCHE, MBA, MSC
ELSA HO, BDS, MBA
THERESA BRASSARD, MA
JACOB FRIEDBERG, MD, FRCS
University of Toronto
Dept of Health Administration
12 Queen's Park Crescent West
Toronto, Ontario M5S 1A8
Canada

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BOOK REVIEWS

Otoneurology and Lateral Skull Base Surgery

Fabrizio Salvinelli and Antonio De la Cruz. Hard cover, illustrated, indexed, 478 pages, 1996. WB Saunders, Philadelphia, Pa, $93.

Purpose: "To reflect the current state of knowledge in this field; especially to make useful for surgical practice."

Contents: Surgeries can be organized into the following: anatomy, facial nerve repair and reanimation techniques, vertigo, acoustic tumor, lateral skull base, and miscellaneous (congenital malformations, cochlear implants, malignancies, drainage of petrous apex lesions, neurophysiological monitoring, complications, and informed consent).

Attributes: Included is significant topographical information with 300 anatomical and intraoperative dissections. (Drawings are hand-drawn, computer-generated designs and are drawn with care and precision.) The authors, drawing from a voluminous source of patients, convey a simplification of the learning and execution of the surgical procedures. "Tips and pitfalls" sections present in most chapters enhance the value of the learning process.

Limitations: Understandably, the material as presented cannot replace careful study of more detailed surgical and anatomical material taken from surgical and cadaveric specimens.

Bibliography: The emphasis of this volume is surgical method. References given at the end of each chapter are of great value to the reader to use for more comprehensive information.

Applicability: Neurotologists, neurosurgeons, and those clinicians interested in the management of cranial base diseases.

DENNIS G. PAPPAS, MD
Birmingham, Alabama

Acoustic Tumors Diagnosis and Management

William F. House, Charles M. Luetje, and Karen Jo Doyle, editors. Hard cover, illustrated, indexed, 359 pages, 1997, 2nd ed. Singular, San Diego, Calif, $125.

Purpose: An update on diagnosis and management of acoustic tumors.

Contents: Can be divided into the following sections: history of acoustic tumor surgery, pathology, evaluation of the acoustic tumor patient, surgical management, postoperative management, and general topics such as neurofibromatosis 2 and stereotactic radiosurgery.

Attributes: This edition (second) examines those recent developments and explores new areas of the acoustic tumor. Important issues today in diagnosis are utilization of auditory brain stem response (this test is surveyed, as well as the current status of hearing preservation) and an overview of the relatively new area of gamma knife treatment. This text demonstrates the state of the art in acoustic pathology and treatment.

Bibliography: Well referenced with contemporary sources.

Applicability: Practicing neurotologists, otolaryngologists, residents, audiologists, and neurosurgeons will benefit most.

DENNIS G. PAPPAS, MD
Birmingham, Alabama

Revision Otologic Surgery

Vincent N. Carrasco and Harold C. Pillsbury III, editors. Hard cover, illustrated, indexed, 203 pages, 1997. Thieme, New York, NY, $89.

Purpose: To deal only with recurrent and recalcitrant otologic and neurotologic disease. Attempts are made to answer preventable causes of failure and that which needs to be done to effect cure.

Contents: Can be divided into revision operations that concern the middle ear (tympanoplasty, stapedectomy, mastoid), and that of the labyrinth (cochlear implantation, surgery of vertigo and skull base). A chapter ends the text on imaging of the postoperative temporal bone.

Attributes: The authors have presented ideas and information to aid the otologic surgeon in a variety of surgical situations. This includes descriptions of pertinent procedural techniques and the most recent of surgical technology such as lasers and ossicular prosthetics. Although there is not a great deal of new technology and much of the revision surgery material has been published in journals, at least the text is compiled and readily available to the reader.

Limitations: Some authors elaborated on primary surgical procedures and did not adhere to the purpose of the book, revision surgery.

Applicability: This text is a valuable reference to otolaryngologists and students, especially residents in otolaryngology, who need to consider those pitfalls that prevent good results.

DENNIS G. PAPPAS, MD
Birmingham, Alabama

Infectious Diseases and Antimicrobial Therapy of the Ears, Nose and Throat

Jonas T. Johnson and Victor L. Yu, editors. Hard cover, illustrated, indexed, 641 pages, 1997. WB Saunders, Philadelphia, Pa, $175.

Purpose: To facilitate the diagnosis and treatment of patients with infectious diseases of the ears, nose, and throat.

Contents: General principles, classification of antimicrobial agents, microorganisms, syndromes, special problems, antibiotic prophylaxis.

Attributes: A comprehensive overview that facilitates the understanding of the microbes important to infections of the ears, nose, and throat. In such a compilation, the index serves as a bridge between diagnosis, etiologic agent, and treatment. This extensive index is well appreciated in this volume, and tends to eliminate, for the reader, general information such as basic anatomy, diagnosis, etc, that is so commonly present in other texts.

Bibliography: All chapters are well-referenced and contemporary sources.

Applicability: This book is well suited for both the general otolaryngologist and the non-otolaryngologist who seeks a good shelf reference.

DENNIS G. PAPPAS, MD
Birmingham, Alabama

Ann Otol Rhinol Laryngol 107:1998

IMMUNOLOGIC ASPECTS OF FACIAL NERVE PARALYSIS INDUCED BY HERPES SIMPLEX VIRUS INFECTION IN MICE

NAOHITO HATO, MD

YASUO HITSUMOTO, MD
NOBUMITSU HONDA, MD
SHINGO MURAKAMI, MD
NAOAKI YANAGIHARA, MD

EHIME, JAPAN

The immunologic aspects of facial nerve paralysis due to herpes simplex virus type 1 (HSV-1) infection were investigated in a mouse model system. Half of the 4- to 5-week-old mice developed facial nerve paralysis, whereas none of the 6-week-old mice died or developed facial nerve paralysis on inoculation with HSV-1. Six-week-old mice showed significantly higher titers of anti–HSV-1 neutralizing antibody than did 4-week-old animals. Passive transfer of either anti–HSV-1 antibody or HSV-1–immunized splenic T cells into 4-week-old mice 3 hours after HSV-1 inoculation prevented development of facial nerve paralysis and death, whereas such transfers 48 or 96 hours after HSV-1 inoculation did not prevent or exacerbate facial nerve paralysis. These results demonstrate that the age and the immunologic potency of mice are closely related to the pathogenesis of facial nerve paralysis. That facial nerve paralysis developed even in 6-week-old mice whose T-cell function was suppressed with anti-CD3 antibody suggests that virus-induced cellular demyelination is unlikely as a cause of facial nerve paralysis in this animal model.

KEY WORDS — antibody, facial nerve paralysis, herpes simplex virus, specific immunity.

INTRODUCTION

Herpes simplex virus type 1 (HSV-1) is a neurotropic virus primary infection that subsides to latency in the sensory ganglia after recovery. Latent HSV-1 infection and its reactivation in the geniculate ganglion of the facial nerve have been strongly suggested as a cause of the idiopathic facial nerve paralysis known as Bell's palsy.[1-4] Recently, we succeeded in making an animal model of transient homolateral facial nerve paralysis by inoculating HSV-1 into the auricle[5,6] and detecting HSV-1 genomes in the clinical samples of endoneurial fluid or posterior auricle muscle obtained during decompression surgery of Bell's palsy patients.[7] However, the pathologic mechanism of facial nerve paralysis remains unclear. Autoimmune reaction against myelin components is a possible cause of peripheral demyelination such as Guillain-Barré syndrome and multiple sclerosis.[4,8,9] The immune system in Bell's palsy was also thought to play a pivotal role in the pathogenesis of the facial nerve paralysis.[10-12] Thus, it is worthwhile studying the relationship between the development of facial nerve paralysis by HSV-1 infection and immunologic potency. The purpose of this study was to clarify whether and how age and specific immunity against HSV-1 are related to the pathogenesis of facial nerve dysfunction in the mouse model system.[5,6]

METHODS

Virus and Animals. The KOS strain of HSV-1, prepared in Vero cells and plaque-titrated at 6.7 × 10^7 plaque-forming units (pfu) per milliliter, was used. Three- to 6-week-old female specific pathogen–free BALB/cAJcl mice were purchased from Clea Japan, Inc (Tokyo, Japan). Four-week-old mice were used as the recipients of passive immunization to study the effects of anti–HSV-1 antibody or immunized T cells on the pathogenesis of facial nerve paralysis. Six-week-old mice were used for immunosuppressive study to clarify whether or not cellular immunity was involved in the pathogenesis of facial nerve paralysis. All animals were cared for in our Laboratory Animal Center, and studies were carried out in compliance with the Guide for Animal Experimentation at Ehime University School of Medicine.

Virus Inoculation and Evaluation of Facial Nerve Paralysis. Following anesthesia by intraperitoneal injection of sodium pentobarbital (50 mg/kg), virus was inoculated into the posterior aspect of the auricle on day 0. The surface of the auricle was scratched 20 times with a 27-gauge needle before HSV-1 inoculation, in a modification of the method of Hill et al.[13] Twenty-five microliters of virus solution (1.7 × 10^6

From the Departments of Otolaryngology (Hato, Honda, Murakami, Yanagihara) and Clinical Laboratory Medicine (Hitsumoto), Ehime University School of Medicine, Ehime, Japan. This research was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, 1994 (6454488), Japan.

CORRESPONDENCE — Naohito Hato, MD, Dept of Otolaryngology, Ehime University School of Medicine, Shigenobu-cho, Onsen-gun, Ehime, 791-02, Japan.

TABLE 1. INCIDENCE OF FACIAL NERVE PARALYSIS AND MORTALITY OF MICE INOCULATED WITH HSV AT VARIOUS AGES

Age (wk)	*Mice With Facial Paralysis*		*Mice That Died*	
	No.	*%*	*No.*	*%*
3	2/20	10	16/20	80
4	13/26	50*	4/26	15*
5	9/20	45*	2/20	10*
6	0/12	0	0/12	0

HSV — herpes simplex virus.

*Significantly different from 3-week-old mice ($p < .05$).

pfu) was placed on the right auricle, and the same volume of phosphate-buffered saline (PBS) was placed on the left. After virus inoculation, blink reflex and vibrissae movements were carefully observed daily so as to detect and evaluate facial nerve paralysis. The blink reflex was elicited by blowing air into the eye with a 5-mL syringe with an 18-gauge needle.

Titration of Anti–HSV-1 Antibody. Mice were partially bled from the retro-orbital plexus with capillary tubes. The separated sera were immobilized by incubation for 30 minutes at 56°C. Fifty microliters of each serum diluted serially was reacted with 50 µL of HSV-1 solution containing 100 pfu virus for 1 hour at 37°C. Then, each solution was added to a monolayer of Vero cells in a 96-well microplate. The anti–HSV-1 antibody titers are expressed as the reciprocal of the dilution of the serum that gave 50% inhibition of cytopathic effect.

Passive Immunization of Anti-HSV-1 Antiserum and Immunized Splenic T Cells. Mice older than 6 weeks were immunized twice on the 12th and 5th days prior to sacrifice, by intraperitoneal injection of 1.7×10^6 pfu of HSV-1. Then, blood and spleen were removed aseptically. The immunoglobulin (Ig) G fraction was obtained by protein G (Pharmacia, Uppsala, Sweden) from the immunized sera and had an anti–HSV-1 titer of 512-fold dilution. Erythrocytes were removed by treating the spleen cells with 0.83% (wt/vol) ammonium chloride solution buffered with Tris (pH 7.2). After washing, cells were suspended in balanced salt solution at 1.25×10^7 cells per milliliter. Splenic T cells were enriched according to the panning method of Engelman et al.[14] Briefly, Petri dishes (Nerbe Plus, Winsen, Luhe, Germany) were coated with 8 mL of 0.25 mg/mL mouse IgG in PBS at 4°C overnight. After washing once with PBS, dishes were incubated with 8 mL of 0.36 mg/mL rabbit IgG fraction containing anti-mouse IgG for 2 hours at room temperature. After washing, 8 mL of the spleen cell suspension (1×10^8 cells) was poured into the mouse IgG-rabbit anti-mouse IgG-coated Petri dishes and incubated for 40 minutes at room temperature. The nonadherent cells were then gently aspirated followed by washing with medium. These cells were then used as the T cell–enriched fractions. A purity of 80% was achieved as assessed by anti-Thy1.2 staining using flow cytometry (EPICS Profile, Coulter, Hialeah, Fla). Four-week-old mice were either injected intraperitoneally with 0.1 mL of anti–HSV-1 antiserum or injected intravenously with 1×10^7 immunized splenic T cells 3 hours, 48 hours, or 96 hours after HSV-1 inoculation.

Immunosuppression of Anti-CD3 Antibody Injection. Six-week-old mice were intraperitoneally injected with a dose of 50 to 200 µg of anti-CD3 antibody before HSV-1 inoculation. We used 145-2C11 monoclonal antibody for the anti-CD3 antibody; it was obtained by growing hybridoma cells provided by Dr A. J. Bluestone (University of Chicago) with the protein G. Mice administered this antibody are both immunosuppressed and mitogenic to T cells.[15] We measured the T cell rate in peripheral mononuclear cells 48 hours and 9 days after the antibody injection with flow cytometry; the rate was below 5% for all concentrations.

Histopathologic Examination. Three nonimmunized and 3 immunosuppressed mice were painlessly sacrificed on the first day of facial paralysis onset to verify the histopathology of the facial nerve. After transcardial perfusion with 10% formaldehyde in phosphate buffer, the bilateral facial nerves were dissected and embedded in paraffin. The specimens were sectioned, stained with hematoxylin and eosin, and then observed under a light microscope.

Statistical Analysis. A nonparametric Mann-Whitney U test was used for analysis of antibody titers, and Fisher's exact probability test was used to determine the significance of mortality and the incidence of facial nerve paralysis.

RESULTS

Age-Dependent Resistance of Facial Nerve to HSV-1. The incidence of facial nerve paralysis and mortality apparently depended on the age of the mouse. Eighty percent of the 3-week-old mice died, and 10% developed facial nerve paralysis following the inoculation of HSV-1. About half of the 4- to 5-week-old mice developed facial nerve paralysis, and 10% to 15% died. In contrast, none of the 6-week-old mice died or developed facial nerve paralysis (Table 1). The incidence of mortality in 3-week-old mice was significantly higher than that in 4- to 6-week-old mice ($p < .05$), and the incidence of facial nerve paralysis in 4- or 5-week-old mice was significantly higher than those of animals inoculated at 3 or 6 weeks old ($p < .05$). Six-week-old mice showed

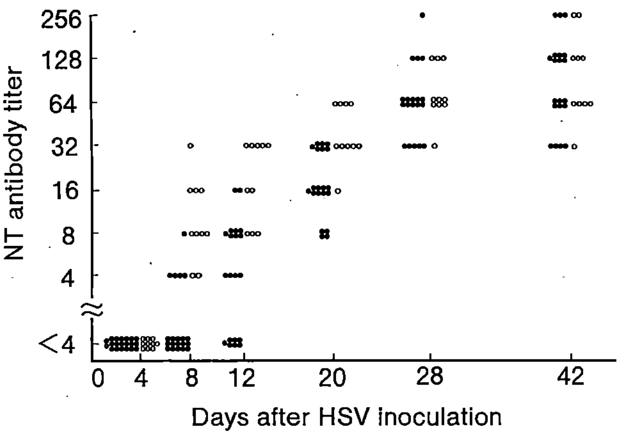

Fig 1. Differences in neutralizing (NT) antibody titers in 4-week-old (filled circles) and 6-week-old (empty circles) mice after herpes simplex virus (HSV)–1 inoculation. Neutralizing antibody titers were negative in all animals before fourth day postinoculation, but became positive by day 20, with titers ranging from 8 to 64. Six-week-old mice showed significantly higher titers of anti–HSV-1 antibody than 4-week-old mice on days 8, 12, and 20 (p < .01).

significantly higher titers of anti–HSV-1 neutralizing antibody than those 4 weeks old (p < .01) during the period between 8 and 20 days postinoculation (Fig 1). The 4-week-old mice with facial nerve paralysis had slightly lower anti-HSV antibody titers than animals of the same age without facial nerve paralysis (Fig 2). The difference between them was significant only in the sera obtained on day 12 (p < .05).

Effect of Passive Immunization on Development of Facial Nerve Paralysis and Mortality. Passive transfer of either anti–HSV-1 antibody or HSV-1–immunized splenic T cells into 4-week-old mice 3 hours after HSV-1 inoculation prevented development of facial nerve paralysis and death (p < .05), whereas transfer 48 hours or 96 hours after HSV-1 inoculation did not decrease the incidence of either facial nerve paralysis or mortality (Table 2). Further, neither an improvement nor a profound effect on the degree of facial nerve paralysis was recognized in the animals that received passive immunization 48 hours or 96 hours after inoculation of HSV-1, compared to the controls.

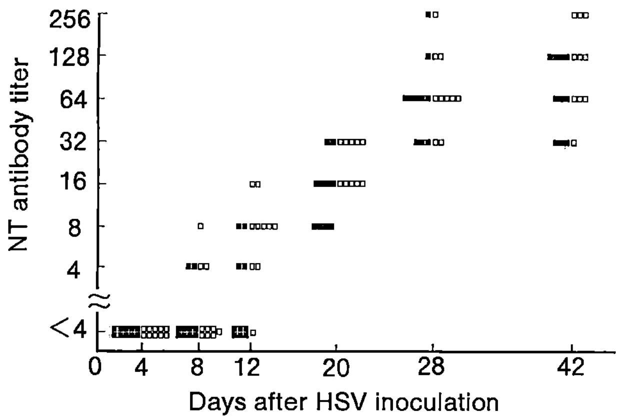

Fig 2. Changes in anti–HSV-1 neutralizing antibody titers in 4-week-old mice with facial nerve paralysis (filled squares) and without facial nerve paralysis (empty squares). All mice had detectable anti–HSV-1 antibody titers at over 32-fold dilution after 28 days. Anti–HSV-1 antibody titers of mice without paralysis were significantly higher than those of mice with paralysis only on day 12 (p < .05).

TABLE 2. EFFECTS OF TRANSFUSED ANTI-HSV ANTIBODY AND IMMUNIZED T CELLS ON INCIDENCE OF FACIAL NERVE PARALYSIS AND MORTALITY IN 4-WEEK-OLD MICE AFTER HSV INOCULATION

	Time After Infection (h)	Mice With Facial Paralysis		Mice That Died	
		No.	%	No.	%
Control		13/26	50	4/26	15
Anti-HSV antibody	3	0/8	0*	0/8	0*
	48	3/8	38	1/8	13
	96	5/10	50	1/10	10
Immune T cell	3	0/8	0*	0/8	0*
	48	4/8	50	1/8	13
	96	5/12	41	1/12	8

*Statistically significant (p < .05).

Effect of Immunosuppression on Development of Facial Nerve Paralysis and Mortality. Sixty-three percent of the mice injected with the anti-CD3 antibody died within 10 days following the inoculation of HSV-1. Thirty-one percent of the 6-week-old immunosuppressed mice developed facial nerve paralysis, but none of the 6-week-old nonimmunized mice developed facial nerve paralysis (Table 3). Two paralyzed mice died 3 to 10 days after facial nerve paralysis.

Histopathologic Findings. In the nonimmunized mice with facial nerve paralysis, severe mononuclear cell infiltration and vacuolar changes were observed in the facial nerve only on the paralyzed side (Fig 3A). In contrast, mononuclear cell infiltration was mild in mice treated with anti-CD3 antibody, even though vacuolar changes were seen.

TABLE 3. INCIDENCE OF FACIAL NERVE PARALYSIS AND MORTALITY OF IMMUNOSUPPRESSED 6-WEEK-OLD MICE INOCULATED WITH HSV

	Mice With Facial Paralysis		Mice That Died	
	No.	%	No.	%
Control	0/12	0	0/12	0
Anti-CD3 antibody	5/16	31*	10/16	63*

*Statistically significant (p < .05).

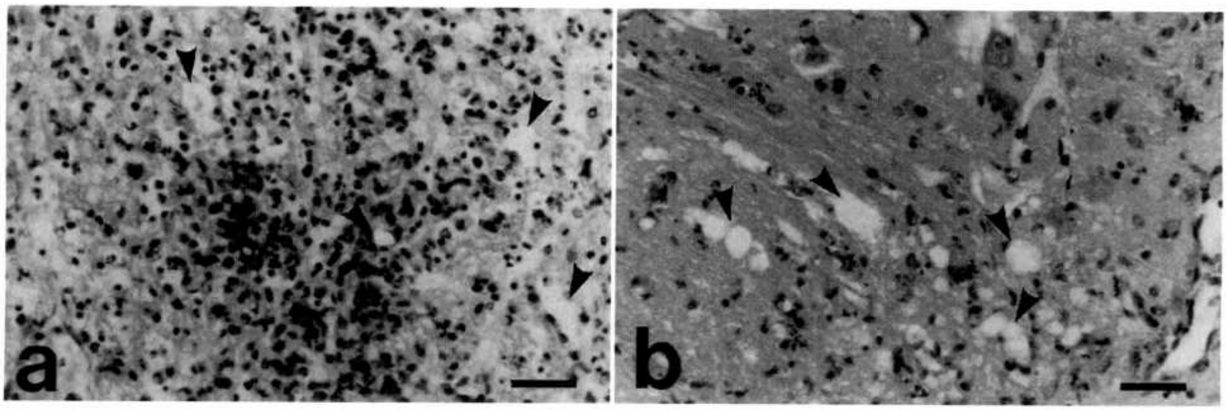

Fig 3. Histopathologic findings of facial nerve of a) nonimmunized and b) immunosuppressed mice having facial paralysis (8 days after inoculation with HSV-1; H & E; bars — 50 µm). a) Note severe mononuclear cell infiltration and vacuolar degeneration (arrowheads) of nonimmunized mice. b) Note mild mononuclear cell infiltration and vacuolar degeneration (arrowheads) of immunosuppressed mice.

DISCUSSION

The present study demonstrates that the development of facial nerve paralysis by HSV-1 infection depends on the age of the mouse at inoculation. The high mortality rate of 3-week-old mice and the high resistance to both development of facial nerve paralysis and mortality of 6-week-old animals may reflect decreasing susceptibility to HSV-1 infection with growth. Similar differences in susceptibility to HSV-1 infection, depending on not only age but also strain of mouse, have been reported.[16] Many factors, including macrophages, natural killer cells, interferon, cellular immunity, and humoral immunity, are known to contribute to resistance to HSV-1 infection.[17-20] Although all these factors may also influence the development of facial nerve paralysis, the immunologic potency that matures with age seems likely to play a significant role. This was supported by the evidence that 4-week-old mice produced lower titers of anti–HSV-1 antibody at the early stage of HSV-1 infection compared to those inoculated at 6 weeks old (Fig 1). Even in the 4-week-old mice, those without facial nerve paralysis had higher anti–HSV-1 antibody titers than those with paralysis (Fig 2).

The results of passive immunization performed 3 hours after inoculation into 4-week-old mice also demonstrated the ability of specific antibodies and immunized T cells to prevent the development of facial nerve paralysis (Table 2). Twenty percent of 4-week-old mice still developed facial nerve paralysis following inoculation with one-hundredth (1.6×10^4 pfu) of the usual dose of HSV-1 (data not shown). Thus, greater than 99% of the inoculated viruses lost their infectivity as a result of passive immunization with either the specific antibodies or specific T cells.

Our results raise the question of why the passive immunization had no capability to prevent facial nerve paralysis when performed later than 48 hours postinoculation. This can be explained, presumably, by the timing of viral infection of the facial nerve and the presence of the blood-brain or blood–peripheral nerve barrier, which prevents circulation of conventional antibodies and lymphocytes from the peripheral immune system.[21] As previously reported, HSV-1 genomes were detectable in the facial nerve as early as 3 days postinoculation and facial nerve paralysis appeared around day 7.[5,6] Thus, the demyelination occurs between days 3 and 7. Once the virus infects the facial nerve or brain stem, passive immunity might fail to attack the virion or virus-infected cells unless the blood-brain or blood–peripheral nerve barrier is broken down. The central nervous system is suggested to have a unique immunologic function and interacts with the peripheral immune system.[22] However, this function may not be able to prevent HSV-1 infection.

The precise mechanism of demyelination — direct viral injury or viral-induced cellular demyelination — is still unknown. However, facial nerve paralysis developed even in 6-week-old mice whose T cells were suppressed by anti-CD3 antibody. Mononuclear cell infiltration in the facial nerve of the immunosuppressed mice was milder than that in the nonimmunized mice. However, vacuolar changes in the facial nerve were seen in both immunosuppressed and nonimmunized mice, and facial palsy developed in both groups of mice. Therefore, viral-induced cellular demyelination is unlikely as a pathologic mechanism of facial nerve paralysis. Moreover, passive immunization of T cells did not produce a higher rate of paralysis or more profound paralysis than in the controls.

Bell's palsy is thought to occur during the course of HSV-1 latency in the geniculate ganglion. Therefore, the mechanism of facial palsy in the primary infection may not be the same as in the reactivation process. If we could reactivate latently infected virus and produce facial nerve paralysis again in the animal model, this might provide clues not only as to the pathologic mechanism of Bell's palsy, but also as to the interaction between the central nervous immune system and virus reactivation. Further investigations to elucidate the immunologic links to virus reactivation and facial nerve damage are currently under way in our laboratory.

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CURRENT EVALUATION OF PSEUDOHYPACUSIS: STRATEGIES AND CLASSIFICATION

WILLIAM W. QIU, MD, MA
SHREVEPORT, LOUISIANA
FRED J. STUCKER, MD
SHREVEPORT, LOUISIANA
SHENGGUANG S. YIN, MD
SHREVEPORT, LOUISIANA
LOUIS W. WELSH, MD
JENKINTOWN, PENNSYLVANIA

Some cases of pseudohypacusis may involve medicolegal aspects and require a confirmed and quantitative diagnosis. These challenging cases must be identified, and then evaluated with basic audiologic and sophisticated electrophysiologic tests. Data on 64 patients with pseudohypacusis collected over a 4-year period are reported. A classification system was developed from an analysis of these cases and is presented for clinical evaluation and diagnosis. In many cases, conventional audiologic evaluation involving pure tone and speech audiometry may be adequate and sufficient for diagnosis. In more complex cases, evoked otoacoustic emissions (EOAEs) and auditory brain stem responses (ABRs) are needed for confirmation of peripheral auditory sensitivity. We found that EOAEs were the most rapid, economical, and objective method, and confirmed the diagnosis of hearing loss in 78.1% of cases. Fifteen percent of subjects required ABRs to substantiate the diagnosis. The reliability of basic audiologic tests based on previous clinical investigations and data from the literature are discussed. We conclude that a thorough knowledge and understanding of pseudohypacusis is essential to verify the existence of pseudohypacusis, to determine its type, and to quantify the auditory thresholds.

KEY WORDS — auditory brain stem response, evoked otoacoustic emissions, hearing loss, pseudohypacusis.

INTRODUCTION

Pseudohypacusis has been defined as functional or nonorganic hearing loss that cannot be explained on the basis of an organic condition or disorder. The basis of pseudohypacusis can be conscious (malingering) or unconscious (psychogenic).[1,2] A patient with pseudohypacusis may demonstrate a false or exaggerated hearing loss that varies in degree, nature, and laterality, such as a unilateral profound hearing loss or bilateral mixed hearing loss. In an uncooperative patient, hearing thresholds obtained may be the results of malingering, simulation, or exaggeration. Pseudohypacusis is encountered fairly often in clinical practice. The prevalence of pseudohypacusis in adults is estimated to range from 8% to 45% in the military, 15% to 20% in referrals from the Veterans Administration, and about 5% to 7% in children.[3-5] On the other hand, the clinician may encounter patients who wish to conceal a handicap and feign better thresholds in the presence of genuine hearing loss. This condition is often found in recruits to the armed forces who are eager to qualify for service even with a hearing loss. Feigning good hearing is also seen in job applicants such as policemen, firefighters, teachers, and physicians during hearing screening. Although the diagnosis and management is similar for these latter cases, this problem is usually not included in discussions of pseudohypacusis.

In general, it is not difficult for an audiologist to detect pseudohypacusis using conventional audiologic procedures in most cases. However, some cases of pseudohypacusis have medicolegal implications and require a timely diagnosis.[6] Other cases of pseudohypacusis are associated with psychiatric or psychological problems.[1,7] The process of diagnosis can be time-consuming, especially as additional tests may be needed to supplement the routine test battery.

Despite the variety of test methods available, pseudohypacusis may present a challenging diagnostic problem. Diagnosing pseudohypacusis requires sufficient knowledge of the disorder so that an appropriate evaluation is employed to accurately assess auditory status. Standard audiologic evaluations may include audiometry, tympanometry, and the acoustic reflex (AR). Other tests reported in the literature to aid in diagnosis have included the Bekesy test,[8] the Lombard test, and delayed auditory feedback.[9] The Stenger test is widely applied for evaluation of unilateral or asymmetric pseudohypacusis.[2] As technology has developed, more advanced audiologic tests have been applied in the evaluation of pseudohypacusis, including the auditory evoked brain stem response (ABR),[10,11] electrocochleography,[12,13] and evoked otoacoustic emissions (EOAEs).[14] These new techniques provide objective information to aid

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From the Department of Otolaryngology–Head and Neck Surgery, School of Medicine of Louisiana State University, Shreveport, Louisiana.
CORRESPONDENCE — William W. Qiu, MD, MA, Dept of Otolaryngology–Head and Neck Surgery, LSU Medical Center in Shreveport, POB 33932, 1501 Kings Highway, Shreveport, LA 71130.

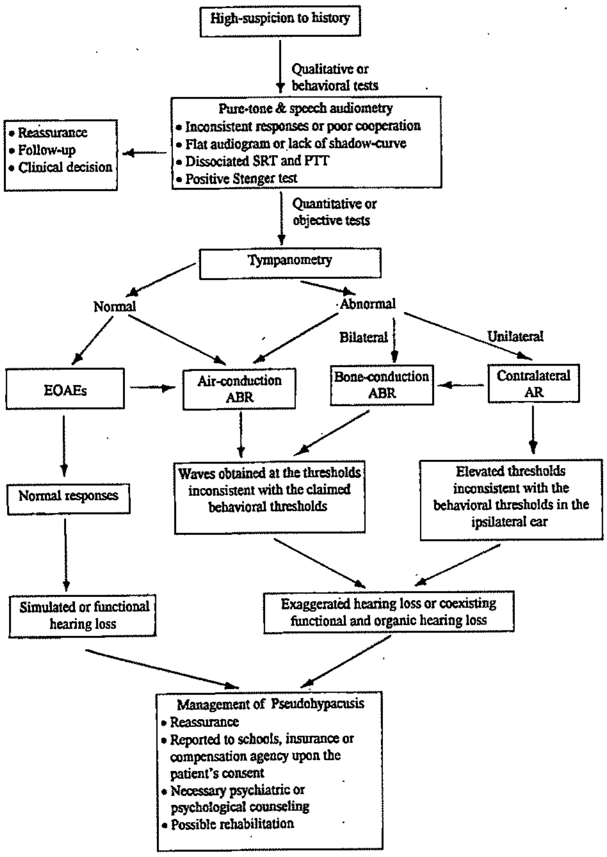

High-suspicion to history
Qualitative or behavioral tests
Pure-tone & speech audiometry
• Inconsistent responses or poor cooperation
• Flat audiogram or lack of shadow-curve
• Dissociated SRT and PTT
• Positive Stenger test
• Reassurance
• Follow-up
• Clinical decision
Quantitative or objective tests
Tympanometry
Normal
Abnormal
Bilateral
Unilateral
EOAEs
Air-conduction ABR
Bone-conduction ABR
Contralateral AR
Normal responses
Waves obtained at the thresholds inconsistent with the claimed behavioral thresholds
Elevated thresholds inconsistent with the behavioral thresholds in the ipsilateral ear
Simulated or functional hearing loss
Exaggerated hearing loss or coexisting functional and organic hearing loss
Management of Pseudohypacusis
• Reassurance
• Reported to schools, insurance or compensation agency upon the patient's consent
• Necessary psychiatric or psychological counseling
• Possible rehabilitation

Fig 1. Preferred diagnostic and management plan for pseudohypacusis. SRT — speech reception threshold; PTT — pure tone threshold; EOAEs — evoked otoacoustic emissions; ABR — auditory brain stem responses; AR — acoustic reflex.

in the detection and quantification of pseudohypacusis. Classification of pseudohypacusis, however, has not been described fully with these new techniques yet. The clinician should be knowledgeable about these objective tests and apply them efficiently to the different types of pseudohypacusis described herein.

The purpose of this paper is to characterize and classify pseudohypacusis by presenting 64 clinical cases. Strategies of evaluation will be discussed.

METHOD

Subjects and Classification of Pseudohypacusis. Sixty-four patients with pseudohypacusis confirmed clinically and audiologically were included in this study. These patients were referred from the Department of Otolaryngology–Head and Neck Surgery, Louisiana State University Medical Center, in Shreveport, La, over a 4-year period. There were 45 males and 19 females. Their ages ranged from 9 to 65 years old, with an average age of 34.5 years. A classification system, using six types, is described below.

Type 1 is a unilateral simulated hearing loss. The patient presents with a severe to profound hearing loss in one ear, with normal hearing sensitivity in the other ear. The patient's true hearing thresholds eventually are identified to be within normal limits bilaterally. This situation is called pure pseudohypacusis or simulated hearing loss.[12]

Type 2 is a bilateral simulated hearing loss. The patient simulates bilateral hearing loss. The nature of claimed hearing loss may be revealed as mild to profound in degree and mixed or conductive, but rarely sensorineural, in nature. The patient's true hearing thresholds are within normal limits bilaterally.

Type 3 is an exaggerated asymmetric hearing loss. The patient demonstrates significantly asymmetric hearing loss. After repeated evaluation, true thresholds may be defined as a mild sensorineural hearing loss (SNHL). Patients may exaggerate the hearing loss in the impaired ear or in the normal-hearing ear. This type of pseudohypacusis often forces clinicians to order more sophisticated studies for an evaluation of asymmetric hearing loss.

Type 4 is a bilateral exaggerated SNHL or coexistence of bilateral organic and functional SNHL. The patient may demonstrate a bilateral hearing loss with a flat audiometric configuration or a configuration similar to high-frequency SNHL. The functional hearing thresholds are superimposed on the existing mild to mild to moderate organic hearing loss in one ear or both ears.

Type 5 is a unilateral exaggerated conductive or mixed hearing loss, or a combination of functional and conductive hearing loss. The patients usually have a previous history of middle ear injury, or have undergone middle ear surgery in one ear. They display exaggerated conductive hearing loss or, more often, a profound mixed hearing loss.

Type 6 is a bilateral exaggerated conductive or mixed hearing loss. Patients with this type of pseudohypacusis usually demonstrate a history of middle ear infection, trauma, or middle ear surgery. On behavioral audiometry, they reveal no response to pure tone or speech stimuli on each ear.

Instrumentation. Pure tone audiometry and speech audiometry were conducted in a standard double-walled booth with a GSI-10 audiometer. Tympanometry and AR thresholds were measured with a GSI-33 Middle Ear Analyzer. The ABR testing was performed with a Spirit evoked potential system (Nicolet Inc, Madison, Wis). The ILO88 Otoacoustic Emissions Analyzer (Otodynamics Inc, Hatfield, England), a computer-based EOAEs analyzer system, was applied to measure EOAEs, which were re-

CLASSIFICATION OF PSEUDOHYPACUSIS IN 64 PATIENTS

Type	*Exaggerated or Simulated Hearing Loss*	*True Hearing*	*No. of Cases*	*%*
1	Unilateral simulated hearing loss	Bilateral normal hearing	28	43.7
2	Bilateral simulated hearing loss	Bilateral normal hearing	12	18.7
3	Exaggerated unilateral moderate to profound hearing loss	Mild unilateral SNHL	8	12.5
4	Exaggerated bilateral moderate to profound hearing loss	Mild bilateral SNHL	10	15.6
5	Exaggerated unilateral conductive or mixed hearing loss	Mild bilateral CHL	2	3.1
6	Exaggerated bilateral conductive or mixed hearing loss	Mild to moderate CHL	2	3.1
Total			64	100.0

SNHL — sensorineural hearing loss, CHL — conductive hearing loss.

corded in a quiet office. All equipment was calibrated routinely.

Procedures. Figure 1 illustrates a preferred diagnostic and management model for pseudohypacusis. Suspicion is initially aroused due to the referral reason or the history, as described above. A discrepancy in the behavioral thresholds (pure tone threshold and speech reception threshold [SRT]) and/or the results of the Stenger test may suggest pseudohypacusis. If the inconsistent responses or discrepant results continue following reinstruction, a battery of audiologic tests are used for quantitative or objective evaluation. Tympanometry usually can detect conductive components. Normal tympanograms identify the patient as a candidate for EOAEs or the AR test. Bilateral abnormal tympanograms lead to measurement using air and/or bone conduction ABR. If the tympanogram is abnormal unilaterally, the contralateral AR test or ABR is conducted. Such diagnostic evaluation should be carried out in the most economical and reliable way possible, keeping in mind the purpose of the evaluation.

RESULTS

The Table summarizes the common types of pseudohypacusis in 64 patients confirmed by objective audiologic evaluations and other otolaryngological examinations. These patients were identified from 4,720 patients who received audiologic evaluation in our clinic over the 4-year period from 1992 to 1996. Thus, the estimated prevalence of pseudohypacusis was 1.35% in this population. The simulated unilateral hearing loss was found to be the most common type of pseudohypacusis (43.7%). An exaggerated conductive or mixed hearing loss accounted for only a small number of patients (6.2%). The following cases illustrate the typical audiologic presentations in verified pseudohypacusis.

Case 1. A unilateral simulated hearing loss (type 1) was confirmed by EOAEs. The patient, a 35-year-old man, reported work-related head injury and hearing loss. He offered normal hearing thresholds in the right ear and no response to pure tones at the limits of the equipment in the left ear without a shadow curve, and a positive Stenger test. Following normal tympanograms, he was tested by EOAEs, which showed normal responses across the frequency spectrum between 1.0 and 5.0 kHz (Fig 2). Reassurance was offered to the patient.

Case 2. The second example is a typical case of bilateral pseudohypacusis or bilateral simulated hearing loss (type 2) in a 9-year-old girl who demonstrated no response to pure tone audiometry. She had a history of child abuse and learning disability. Both AR thresholds and EOAEs were well within normal limits (Fig 3). Following psychiatric counseling, she was tested by ABR, which showed normal latencies of waves I, III, and V at 80 dB normal hearing level (nHL) and the presence of wave V at 35 dB nHL bilaterally. The patient was referred for an evaluation by a speech and language pathologist in this clinic and demonstrated normal speech and language. She then received a diagnosis of bilateral pseudohypacusis or psychogenic deafness. She was referred for psychological rehabilitation.

Case 3. The patient, a 65-year-old woman, presented with a 3-day history of ear pain in the left ear after receiving a blow to her head. On the first evaluation she revealed a total hearing loss in the right ear with normal hearing thresholds in the left ear, and normal tympanograms in both ears. The AR test demonstrated elevated thresholds at high frequencies in the left ear. The EOAEs showed responses only at the low frequency (1,000 Hz) with reduced or absent responses at high frequencies in the right ear. Figure 4 illustrates audiologic findings of an exaggerated, asymmetric hearing loss in this patient (type 3). Based on these results, she was estimated to have a mild high-frequency SNHL in the right ear.

Case 4. A 35-year-old man claimed compensation for severe bilateral hearing loss due to industrial noise exposure (type 4). The patient initially demonstrated bilateral severe mixed hearing loss on pure tone audiometry. But tympanometry revealed normal tym-

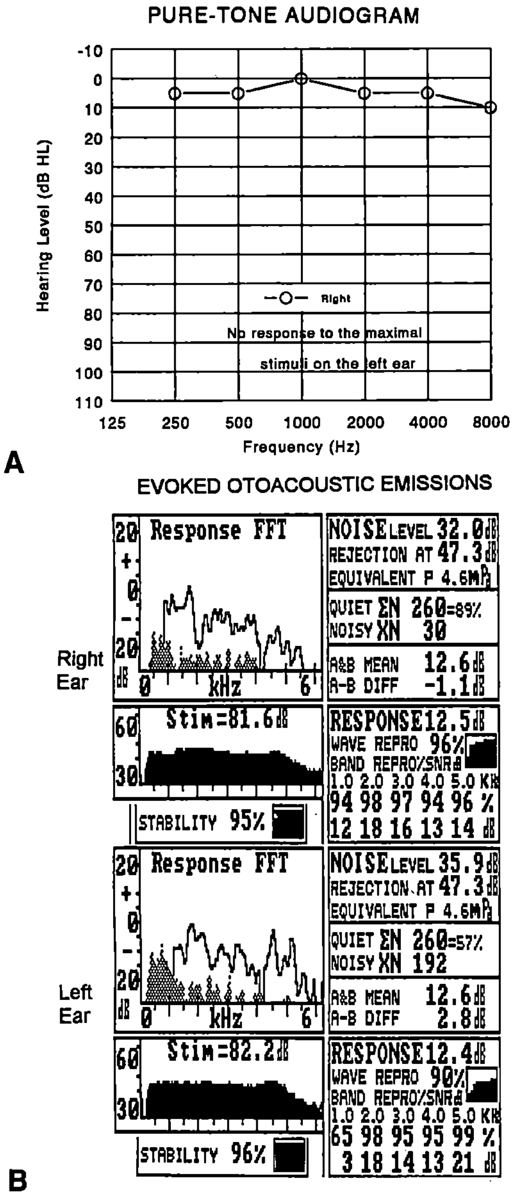


Fig 2. (Case 1) Findings of EOAEs in 35-year-old man with pseudohypacusis in left ear. Patient received head injury in industrial accident and refused to acknowledge hearing any sound at equipment limits, as shown in A) pure tone audiogram. B) EOAEs revealed normal responses bilaterally.

panograms for both ears, and the AR was present at 1,000 Hz bilaterally. The configuration of the pure tone thresholds in both ears was confirmed by the spectrum analysis of EOAEs (Fig 5). Following explanation of the inconsistent results and reinstruction of the testing procedure, he became cooperative in the second pure tone audiometry, which revealed high-frequency SNHL bilaterally.

Acoustic Reflex Measurement

Freq (kHz)	Right Ear		Left Ear	
	Ipsi	contra	Ipsi	contra
0.5	75	85	80	80
1	75	85	75	75
2	80	90	80	80
4	80	90	80	85

A

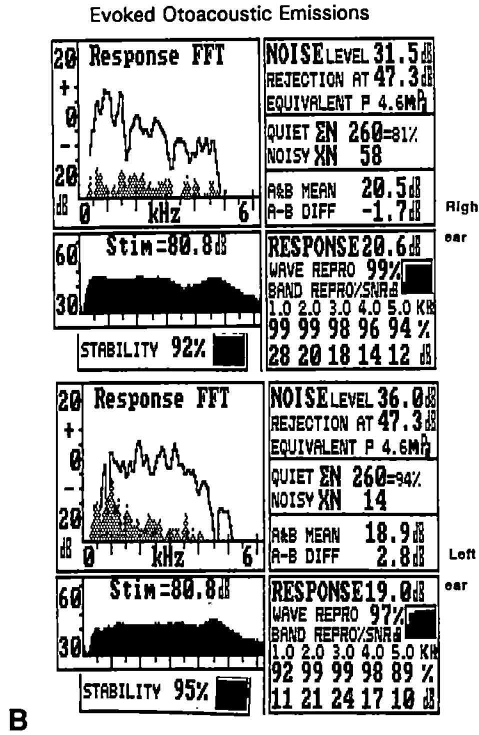
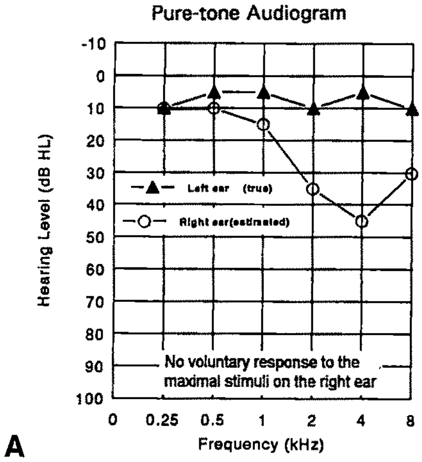


Fig 3. (Case 2) Audiologic data in 9-year-old girl who showed no response to maximal stimuli in pure tone audiometry for both ears. A) Acoustic reflex thresholds and B) EOAEs are within normal limits bilaterally.

Case 5. Figure 6 demonstrates objective audiologic data obtained in an 18-year-old female prisoner who alleged a total hearing loss bilaterally for 1 month (type 4). The patient's history included syphilis and drug abuse for several years. On audiometry, she demonstrated no response to the maximal stimuli of the equipment. Then EOAEs were applied and showed no response in the left ear and a markedly reduced response in the right ear. Testing of the ABR was subsequently conducted and showed recognizable wave V down to 60 dB nHL bilaterally. These results suggest that the patient's peripheral auditory sensitivity for high frequencies was approximately 50 to 60 dB hearing level (HL) for both ears. At a 1-year follow-up, she was recommended for hearing aids.

Case 6. Figure 7 demonstrates typical findings of a unilateral, exaggerated conductive hearing loss in the left ear (type 5). The patient was a 28-year-old man with a 3-day history of traumatic hemotympanum subsequent to a head injury. He showed no audiometric response with a flat tympanogram in the left ear, and normal hearing sensitivity with a normal tympanogram in the right ear. The AR was pres-



Acoustic Reflex Measurement

Freq	Right Ear		Left Ear	
(kHz)	Ipsi	contra	Ipsi	contra
0.5	85	80	80	80
1	85	80	75	85
2	100	85	80	95
4	110	85	85	ab

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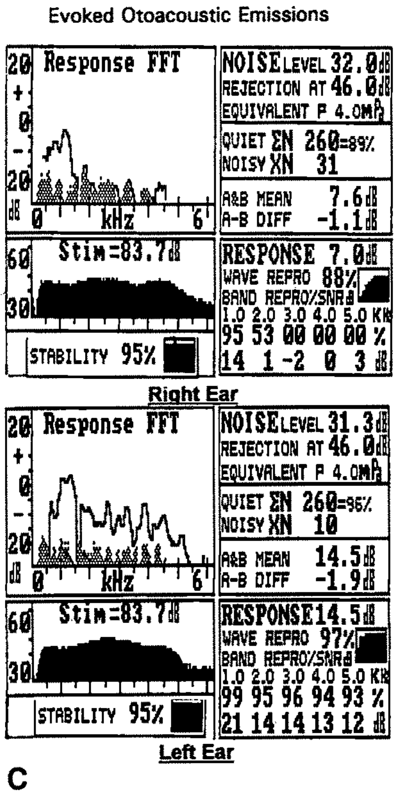


Fig 4. (Case 3) Audiologic results for 65-year-old woman who described total hearing loss in right ear with normal hearing thresholds in left ear as shown in A) pure tone audiogram. B) Acoustic reflex showed elevated thresholds or was absent at 2,000 and 4,000 Hz for ipsilateral and contralateral stimulation in right ear. C) EOAEs revealed responses only at low frequency (1,000 Hz), with reduced or absent responses at high frequencies in right ear, compared to normal responses in left ear. According to these results, mild high-frequency sensorineural hearing loss is therefore estimated in right ear.

ent at normal thresholds ipsilaterally, but was elevated contralaterally when the probe tone was in the right ear. In normal-hearing subjects, the difference between ipsilateral and contralateral AR thresholds is usually within 5 and 10 dB. Hemotympanum associated with a flat tympanogram generally produces at least 15 to 20 dB air conduction hearing loss or an air-bone gap. Therefore, the elevation of 15 to 20 dB in AR thresholds on the stimulation of the left ear, compared with the ipsilateral AR thresholds in the right ear, is likely related to hemotympanum and should suggest a mild conductive hearing loss in the left ear (Fig 7C). The patient's auditory status in the left ear had returned to normal on follow-up 6 weeks after injury.

Case 7. Figure 8 demonstrates the audiologic findings in a 45-year-old man who underwent bilateral mastoidectomies for chronic mastoiditis and cholesteatomas in the past 2 years. The patient claimed a total hearing loss in both ears (type 6) and applied for disability compensation. Considering the patient's middle ear status, he had at least 50 to 60 dB hearing loss (the possible conductive components) for both ears. Air conduction ABR showed questionable peaks at 95 dB nHL with poor waveforms bilaterally. Due to inconclusive information from immittance audiometry and air conduction ABR, he was then tested by bone conduction ABR to confirm his auditory status. As illustrated in Fig 8, wave V was recorded ipsilaterally at 10 to 20 dB nHL in both ears, consistent with the bone conduction pure tone thresholds at 5 to 15 dB HL. Combined with the history of middle ear disease and surgery, it was estimated that the patient's air conduction pure tone thresholds were at most 60 to 70 dB HL (the possible maximal conductive components) at some frequencies for at least one ear. Thus, these results may rule out the presence of bilateral total hearing loss. The patient was referred for possible middle ear reconstruction or bone conduction hearing aids.

DISCUSSION

Compensation claims for occupational noise-induced hearing loss, working accidents, or insurance are commonly seen in clinical practice. Compensation is often directly related to the hearing loss severity; thus, attention must be paid to the accurate quantification of thresholds. The clinical evaluation of any patient suspected of pseudohypacusis should include a degree of skepticism regarding the accuracy of the subjective behavioral tests. Any question concerning the reliability of this information suggests that further confirmation is necessary. Careful assessment by conventional pure tone audiometry may limit the need for further referral. Appropriate application of an audiologic test battery is essential to confirm and quantify the hearing loss. According to our clinical experience and the contemporary knowledge and techniques of audiology, classification of pseudohypacusis into six types was attempt-

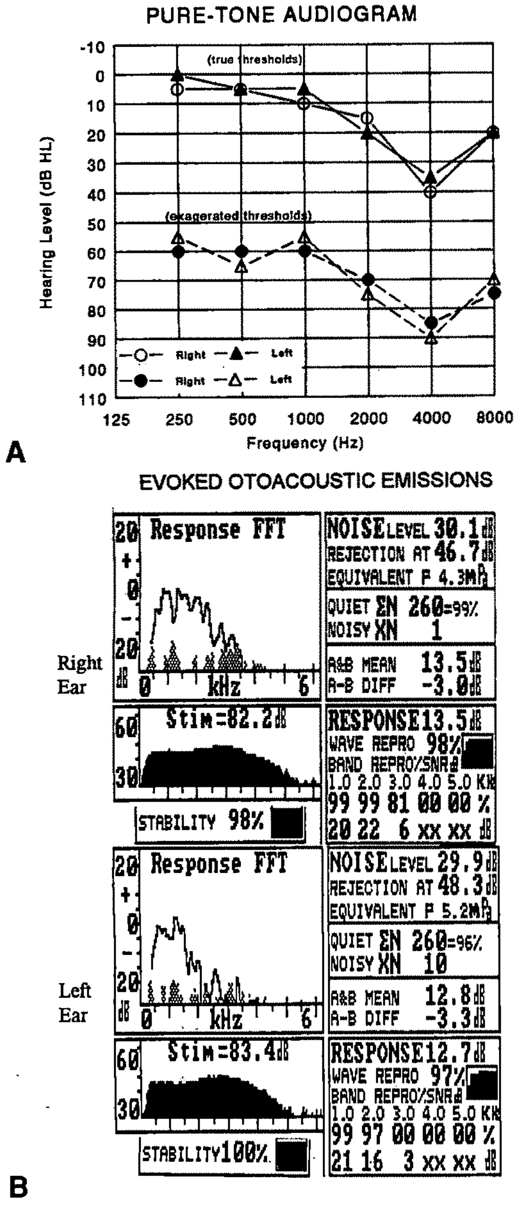


Fig 5. (Case 4) High-frequency hearing loss was confirmed by EOAEs in 35-year-old man who claimed workers' compensation for bilateral severe hearing loss. Note that A) configuration of pure tone thresholds obtained following reinstruction was consistent with B) spectrum of EOAEs.

ed in this study to facilitate clinical diagnosis and management. The classification and the diagnostic paradigm (Fig 1) that we recommend in this investigation are based on the following considerations.

Characteristics of unilateral pseudohypacusis (type 1) include lack of agreement between SRTs and pure tone averages, and a speech or pure tone Stenger test that is usually positive. Another feature is the lack of a shadow curve, especially for bone conduction. Total unilateral hearing loss is more likely to be seen in patients involved in medicolegal context cases.[12] An audiologist should suspect nonorganic hearing loss based on observation of patients' behavior and manifestations of audiometric evaluation mentioned above. This can be supported by means of conventional hearing assessment combined with tympanometry and EOAEs in clinical practice most of the time.

Bilateral pseudohypacusis (type 2) often occurs in patients who demonstrate a flat audiogram and symmetric hearing loss for both ears. The degree of the exaggerated hearing loss varies from mild to totally absent voluntary thresholds. The true hearing thresholds are within normal limits bilaterally. The bone conduction thresholds are usually inconsistent with the correspondent air conduction thresholds. A false air-bone gap may be present.

Previous investigations have indicated that a functional hearing loss is most frequently superimposed on an organic loss rather than on normal hearing sensitivity.[15] With coexistence of organic and functional SNHL in one ear or exaggerated, asymmetric hearing loss (type 3), patients may pretend or exaggerate hearing loss on the opposite side. Patients usually exaggerate their hearing level to a profound or total loss without presence of a shadow curve on the audiogram. Because asymmetric hearing loss often alerts the clinician to rule out a retrocochlear lesion, more sophisticated tests are usually ordered. Testing of EOAEs is valuable initially to confirm the hearing status. The EOAE results may demonstrate normal responses in one ear and reduced responses in the other ear, as shown in Fig 4. Using more than a 10-dB discrepancy between pure tone thresholds and SRTs as a criterion for diagnosis of exaggerated hearing loss, Alberti et al[16] found that 20.3% of 596 patients who failed a hearing screening were correctly diagnosed as pseudohypacusic. They also reported that of 121 exaggerators, 62% were correctly identified by ABR.

For potential monetary gain or for psychiatric reasons, patients may exaggerate their mild to moderate bilateral hearing loss, as shown in Figs 4 and 5. This condition results from the coexistence of functional and organic SNHL in both ears or bilateral exaggerated hearing loss (type 4). This type of functional hearing loss can be evaluated with EOAEs and/or ABR.

Coexistent functional and conductive hearing loss in one ear is also called unilateral exaggerated conductive or mixed hearing loss (type 5). Most of the time, patients present with an ear injury with hemotympanum or tympanic perforation. To confirm the hearing status, multiple tests may be needed. With the exception of inconsistent behavioral hearing loss results and unusually large air-bone gaps (more than

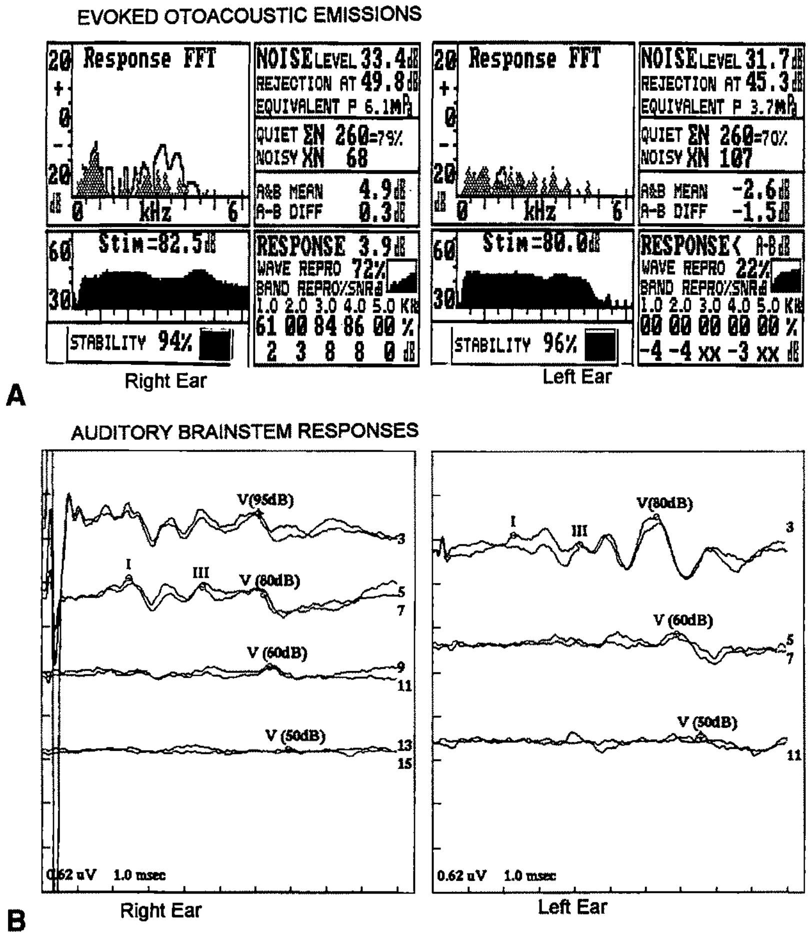
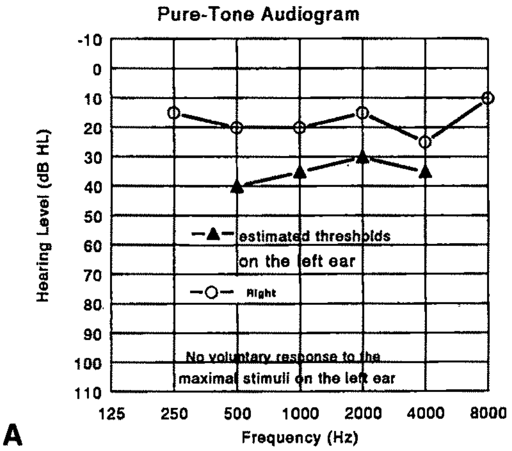


Fig 6. (Case 5) Objective audiologic data obtained from 18-year-old woman who claimed deafness for both ears. She was uncooperative in behavioral hearing testing. A) EOAEs revealed reduced responses in right ear and absent response in left ear. B) Auditory brain stem responses were present at 60 dB normal hearing level bilaterally, consistent with 50 to 60 dB of hearing loss at least for high frequencies.

65 to 70 dB), an AR test can be initially used if patients only present unilateral exaggerated conductive hearing loss with normal hearing and tympanogram in the opposite ear. For example, the contralateral AR may be elicited with the stimulating probe in the malingered ear. The presence of the contralateral AR at 110 to 120 dB HL usually indicates air conduction pure tone thresholds around 40 to 60 dB HL. If the patient's bone conduction thresholds are normal, mild to moderate conductive hearing loss can be confirmed. In addition, ABR can be used in this case if the AR is absent or unsuccessfully obtained. Principato et al[17] presented a case of unilateral conductive hearing loss produced by a nasopharyngeal mass in a 31-year-old man. That patient claimed bilateral hearing loss in an attempt to relate his symptoms to trauma sustained in an automobile accident. The patient was finally confirmed to have normal hearing in the right ear and conductive hearing loss in the left, which was corrected by adenoidectomy 2 months later. This observation suggests that careful differentiation of organic hearing loss from nonorganic hearing loss must be made.

Coexistent functional and conductive hearing loss for both ears (type 6) is also called bilateral exaggerated conductive or mixed hearing loss. Patients usually have a history of middle ear infections or have undergone middle ear surgery in both ears. Occasionally, patients are seeking medical disability or a compensation for failed middle ear surgery, such as tympanoplasty, ossicle chain reconstruction, and stapedectomy. It is most difficult to verify the hearing thresholds with certainty in this type of pseudohypacusis. Due to dysfunctional middle ear status, tympanometry, the AR, and EOAEs do not contribute to the quantification of thresholds. Testing of ABR may be the choice for evaluation of this special case (Fig 7). Air conduction ABR may characteristically show the displacement of the latency-intensity function in the impaired ear to the right of the normal or control ear. In case of substantial mixed hearing loss, air conduction may show no response or an unconfirmed wave at a high level of stimulation. Recent investigations have demonstrated the application of bone conduction ABR for determining the conductive components or hearing loss that cannot be evaluated by traditional methods.[18-20] Electrophysiologic measurement of air-bone gaps by air and bone conduction ABR can be used to estimate the magnitude of conductive hearing loss in the same way as behavioral tests are used.[21] The masking dilemma was like-



Tympanometry

	Type	Canal Vol (ml)	Pressure (dapa)	Admittance (ml)
Right	A	1.2	-45	0.5
Left	B	1.1	-	0

B

Acoustic Reflex Measurement

Frequency (kHz)	Right Ear		Left Ear	
	Ipsi	contra	Ipsi	contra
0.5	80	100	ab	ab
1	80	95	ab	ab
2	80	95	ab	ab
4	85	95	ab	ab

C

Fig 7. (Case 6) 28-year-old man with 3-day history of traumatic hemotympanum resulting from automobile accident presented with no response in left ear by A) pure tone audiometry. B) Tympanometry revealed flat tympanogram (type B) in left ear and type A tympanogram in right ear. C) Acoustic reflexes were present at normal thresholds ipsilaterally, but elevated contralaterally when probe tone was in right ear. Acoustic reflex in left ear was absent for both ipsilateral and contralateral conditions. These results suggest mild conductive hearing loss in left ear.

ly present, not only for pure tone audiometry, but also for the ABR. However, use of masking in bone conduction ABR is complex. Since the ipsilaterally recorded peaks are earlier, more robust, and more repeatable than the contralaterally recorded peaks for bone conduction ABR in clinical testing,[19] the use of masking, or the overmasking or undermasking of the nontest ear, may be neglected, as in case 7. However, the interpretation of bone conduction ABR should be cautious for ear-specific information. Such cases are difficult to evaluate, but fortunately are rare.

Contemporary aspects of audiology have provided new tools for evaluation and diagnosis of pseudohypacusis. For example, EOAEs have been applied as a fast and objective test to confirm the hearing status in patients with pseudohypacusis. If present, EOAEs usually suggest peripheral sensitivity better than 20 to 35 dB HL.[22] Musiek et al[14] reported 5 cases of pseudohypacusis in which EOAEs were used to cross-check the validity of audiologic thresholds. They recommended use of EOAEs in diagnosis of pseudohypacusis. The EOAEs may also provide some frequency-specific information, particularly in noise-induced hearing loss, as shown in Figs 4 and 5. The pattern of spectrum responses of EOAEs often corresponds to the notched pure tone audiograms. In general, normal EOAEs usually confirm normal middle ear and cochlear function, and thus may rule out a moderate to severe hearing loss and the presence of conductive components. The EOAEs have particular value in evaluation of patients with suspected cochlear recruitment. In relatively rare cases,

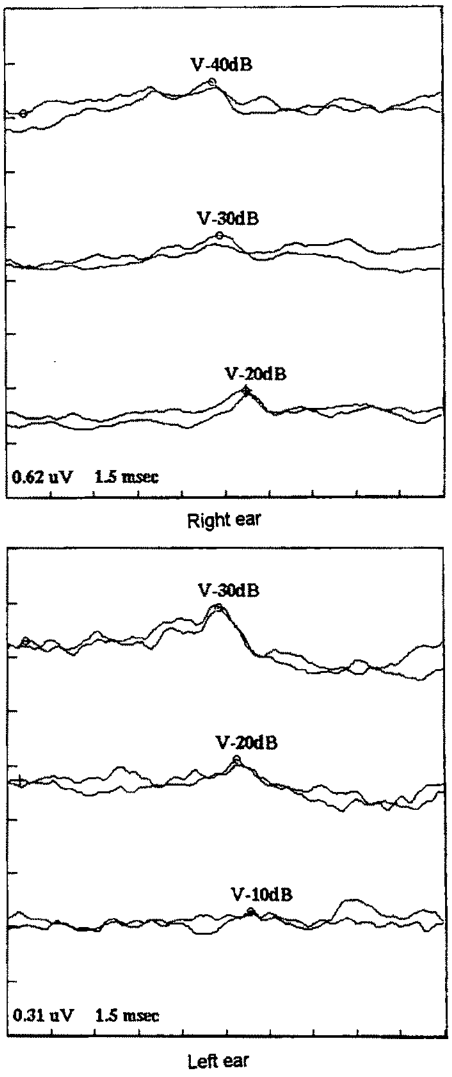


Fig 8. (Case 7) 45-year-old patient who underwent bilateral mastoidectomies for cholesteatomas in past 2 years presented with total bilateral hearing loss and claimed medical disability. He was tested by bone conduction auditory brain stem responses. Wave V was obtained at 20 dB normal hearing level for both ears, consistent with behavioral bone conduction thresholds at 5 to 10 dB hearing level. On basis of history of middle ear disease and surgery, patient's air conduction thresholds are estimated at 50 to 60 dB hearing level for at least one ear.

EOAEs may be present in spite of a severe to profound hearing loss.[23-26]

The ABR, when used and interpreted properly, provides a reliable method of estimating auditory sensitivity that cannot be evaluated by behavioral testing.[18] Currently, the ABR has been proposed as a standard test to verify the behavioral thresholds in nonorganic hearing loss.[10,27,28] However, the ABR is generated by subcortical structures only. Measurement of the ABR can never be considered a true hearing test.[19] It should also be noted that ABR thresholds for clicks usually underestimate low-frequency hearing sensitivity, while tone ABRs or bone-conducted ABRs have more technical demands for stimulus, filter, recording methods, and response interpretation. In addition, the audiologist usually will take 30 to 60 minutes to complete the threshold testing using conventional ABR. In noise-induced hearing loss, which is frequently manifested as a high-frequency loss at 4,000 Hz, ABR with clicks as stimuli may not provide frequency-specific information for a true configuration of hearing thresholds.

An audiologic test battery, instead of a single test, should be applied in the evaluation of pseudohypacusis. A preferred diagnostic and management model is proposed in this study. Acoustic immittance audiometry is a valuable tool in diagnosis of pseudohypacusis (Fig 1). Tympanometry can quickly reveal middle ear status and other conductive components of hearing loss. This test provides important information for an audiologist to select the next test for the diagnosis (Fig 1). In patients with a significant air-bone conduction gap and normal tympanograms, the clinician needs to rule out the possibility of ossicular chain abnormality by the AR or EOAEs. The AR is very useful in determining the presence of a functional hearing loss, especially in types 1 and 2 (simulated unilateral or bilateral hearing loss) or types 4 and 5 (exaggerated unilateral hearing loss).[29,30] A claimed unilateral or bilateral profound hearing loss can be ruled out if the AR is present at a normal stimulation level, which is elicited at 70 to 90 dB sensation level (SL) ipsilaterally and contralaterally. The only exception is the existence of a cochlear or cortical disorder, in which the AR may be present at 25 to 60 dB SL. Thus, the phenomenon of cochlear recruitment may limit the usefulness of the AR test in estimation of thresholds and diagnosis of pseudohypacusis. However, the AR is rarely present at an intensity level less than 15 to 20 dB SL.[31] In addition, an audiologist can readily compare the bilateral cochlear status to estimate whether recruitment is present in the pseudohypacusic ear. In severe SNHL or conductive hearing loss, the AR is absent and provides no quantitative information about hearing sensitivity.

Finally, it must be recognized that some patients with auditory communication difficulty and normal objective audiologic findings may suffer from a central auditory disorder other than pseudohypacusis. In rare cases of cortical or subcortical lesion involving the auditory pathways, the patients may demonstrate substantial behavioral hearing loss but show some combination of normal AR, EOAEs, or ABR tests.[23-26] The clinician should pay attention to the patients' speech understanding ability, and conduct central auditory tests, including middle latency responses, or request neuroradiologic examinations if necessary to rule out any organic central auditory disorder.

CONCLUSION

Basic audiometric tests, including pure tone, speech, and impedance audiometry, suffice for the purposes of detection and evaluation of pseudohypacusis in most cases. The ABR, EOAEs, and other electrophysiological tests may provide further information, and they should be applied when basic audiometric measures fail to accomplish the goal of diagnosis. Appropriate classification of pseudohypacusis and the use of a test battery facilitate the identification and quantitative evaluation of a functional hearing loss. We emphasize that thorough understanding of pseudohypacusis and clinical experience are important in diagnosis and management of pseudohypacusis.

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AGE-RELATED CHANGES IN COCHLEAR BLOOD FLOW RESPONSE TO OCCLUSION OF ANTERIOR INFERIOR CEREBELLAR ARTERY IN MICE

TORU SUZUKI, MD
NAGOYA, JAPAN

TIANYING REN, MD
PORTLAND, OREGON

ALFRED L. NUTTALL, PHD
PORTLAND, OREGON

JOSEF M. MILLER, PHD
ANN ARBOR, MICHIGAN

Previous studies suggest that autoregulation of cochlear blood flow (CBF) may be impaired by aging. In this study, we quantified the capacity of CBF autoregulation in young adult (6-month-old) and aged (21-month-old) CBA mice, to examine the effect of aging on CBF autoregulation. The technique of anterior inferior cerebellar artery (AICA) occlusion was employed to manipulate cochlear perfusion pressure. The CBF was measured by laser Doppler flowmetry during intermittent occlusion of the AICA with a microvascular clamp, which causes an initial decrease in CBF. The autoregulation of CBF was evaluated by quantifying the CBF increase (compensatory dilatory response in the cochlear vessels) during AICA occlusion. Our results demonstrated that autoregulation of CBF was significantly reduced and that collateral vascular function supplying CBF was significantly lower in the aged group. These findings suggest that the aged ear has less capacity to maintain stable blood flow and thus may be more vulnerable to stress factors that affect cochlear function.

KEY WORDS — autoregulation, collateral vascular function, laser Doppler flowmetry, presbycusis.

INTRODUCTION

Human temporal bone studies[1,2] and experimental animal models[3,4] have demonstrated age-related histopathologic changes in the cochlear vasculature, and cochlear blood flow (CBF) alteration is considered one of the etiologic factors of presbycusis. Using the surface dissection microsphere method, Prazma et al[5] reported a significant CBF decrease in gerbils with aging, which supported a vascular theory of presbycusis. Besides these changes in vascular morphology and absolute blood flow value, another possible factor contributing to CBF alteration with aging is change in the capability of the vascular system to control blood flow.

Studies[6,7] have shown strong autoregulatory capability in the cochlear vasculature, which plays an important role in maintaining stable blood flow in the cochlea. In a preliminary study (unpublished), we observed decreased autoregulatory capacity of the cochlear vasculature in aged CBA mice compared to young ones. In that study, interpretation of the results was complex, because changes in the perfusion pressure were elicited by administration of a vasoactive drug and exsanguination. These methods elicit both systemic and local cochlear effects, and thus make it difficult to be confident in the extent to which observed CBF compensatory changes are driven by systemic factors or local (autoregulatory) factors. Ren et al[8] demonstrated that the anterior inferior cerebellar artery (AICA) can be occluded repeatedly without changing vascular reactivity by using a special microclamp system and that AICA occlusion is a suitable local manipulation for investigating autoregulation of the cochlear vascular system.

The purpose of this study was to use the AICA occlusion technique to quantify the autoregulatory capacity of the cochlear vessels in young adult CBA mice (6-month-old) and aged ones (21-month-old) and to examine the effect of aging on CBF autoregulation.

MATERIAL AND METHODS

Animal Preparations. Twenty CBA mice of either sex, ages 6 months ($n = 10$) and 21 months ($n = 10$), obtained from the National Institute of Aging, were used in this study. The animals were housed in Ameri-

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From the Kresge Hearing Research Institute, University of Michigan Medical School, Ann Arbor, Michigan (Suzuki, Nuttall, Miller), the Department of Otorhinolaryngology, Nagoya University School of Medicine, Nagoya, Japan (Suzuki), and the Oregon Hearing Research Center, Oregon Health Sciences University, Portland, Oregon (Ren, Nuttall). This work was supported by National Institutes of Health/National Institute on Deafness and Other Communication Disorders grant R01 DC00105 and the Japan Foundation for Aging and Health. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Michigan.

CORRESPONDENCE — Alfred L. Nuttall, PhD, Oregon Hearing Research Center NRC04, Oregon Health Sciences University, 3181 SW Sam Jackson Park Rd, Portland, OR 97201-3098.

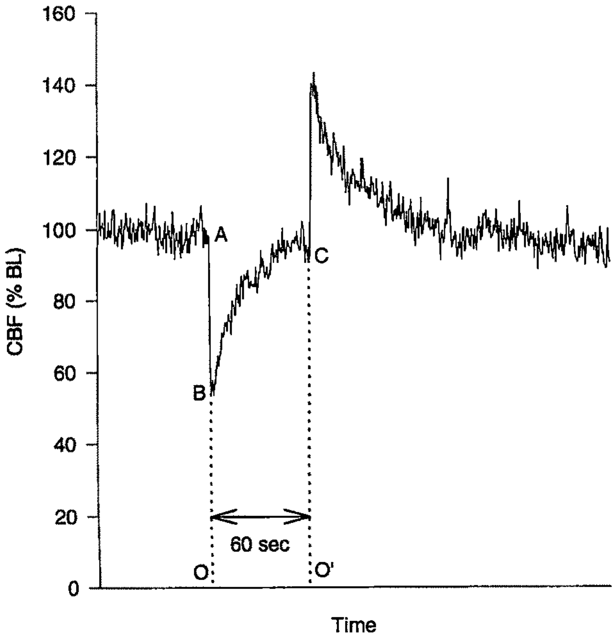

Fig 1. Response of cochlear blood flow (CBF) during 60-second occlusion of anterior inferior cerebellar artery (AICA) in young mouse. To calculate AICA contribution and change in vascular conductance (vascular responsiveness), following measurements are made: A — baseline (BL) of CBF; B — CBF at onset of acute occlusion; C — CBF prior to clamp release. Percent AICA contribution to CBF is indicated by AB. Vascular responsiveness was calculated as follows: Vascular responsiveness = CO'/BO.

can Association for Accreditation of Laboratory Animal Care–approved facilities on a 12:12-hour light-dark cycle initiated at 6:00 AM, with food and water available ad libitum.

Surgical Approach. Anesthesia was induced with ketamine (100 mg/kg, subcutaneously) and xylazine (5 mg/kg, subcutaneously) and maintained by a supplementary half dose of ketamine administered every 30 minutes. Rectally measured body temperature was maintained at 38°C ± 1°C with a heating pad and a lamp. A tracheotomy was performed to ensure free breathing, and the left femoral artery was cannulated with PE-10 tubing for continuous blood pressure (BP) measurement. After the animal's head was firmly fixed in a head holder, the trachea and esophagus were transected, and muscular attachments to the skull base were excised. The base of the skull was carefully drilled with a diamond bur, and the dura was opened to expose the basilar artery and the right AICA. The right tympanic bulla was then exposed by a ventral surgical approach and was opened with a diamond bur. The middle ear mucosa was gently removed with a cotton pledget from the lateral surface of the cochlea.

CBF Measurement and AICA Clamping. To measure CBF, the 0.4-mm diameter probe of a laser Doppler flowmeter (Periflux PF2B, Perimed, Stockholm, Sweden) was placed on the lateral wall of the first turn of the cochlea. Petroleum gel was applied to the probe tip to enhance optimal coupling of the laser light to the cochlea and minimize the accumulation of blood or fluid under the probe. The time constant was set to 0.2 second and the frequency cutoff to 4 kHz.

A custom-designed microclamp[8] was held in a micromanipulator and positioned so that the AICA was between the clamp tips. When the microclamp system was in position, the pressure between the clamp tips was just sufficient to completely occlude the vessel but gentle enough not to damage it even after repeated occlusions. Although there were anatomic variations of the cerebellar arteries, the AICA was easily identified by the following characteristics: 1) it was the largest branch of the basilar artery, between the posterior inferior cerebellar artery and the superior cerebellar artery; 2) it was straight and unbranched inside the surgical field; and 3) clamping caused a significant response in the laser Doppler flowmetry signal. For this study, the vessel could be occluded in the same place up to 7 times without a change in vessel reactivity.

The CBF and BP were recorded simultaneously on a computerized chart recorder with a sampling frequency of 2 Hz.

Experimental Protocol. To observe the time-dependent dynamic response of CBF, the AICA was occluded for 3, 5, 10, 15, 30, 60, and 120 seconds in each animal. The interval between the occlusions was long enough to allow complete CBF recovery. Blood pH, PCO2, and PO2 were determined (STAT Profile 3 blood gas analyzer, Nova Biomedical) from a 0.2-mL arterial blood sample taken at the end of the experiment.

Data Analysis. The AICA occlusion data were analyzed as follows. The baseline (BL) of CBF was determined by averaging the 1 minute prior to occlusion (A in Fig 1). Biological zero flow was determined postmortem at the end of the experiment. The levels of CBF at occlusion (BO) and prior to release (CO') were measured on the basis of percent of BL. The percent decrease of CBF at B (segment AB) in Fig 1 indicates the percentage of AICA contribution to CBF.[8] This percent AICA contribution was determined in each animal.

In this study, autoregulation was defined as the change in vascular conductance that occurred during clamping (O to O', Fig 1). Vascular conductance is defined as the ratio of flow to perfusion pressure. At times O and O' (Fig 1), perfusion pressure is equal,

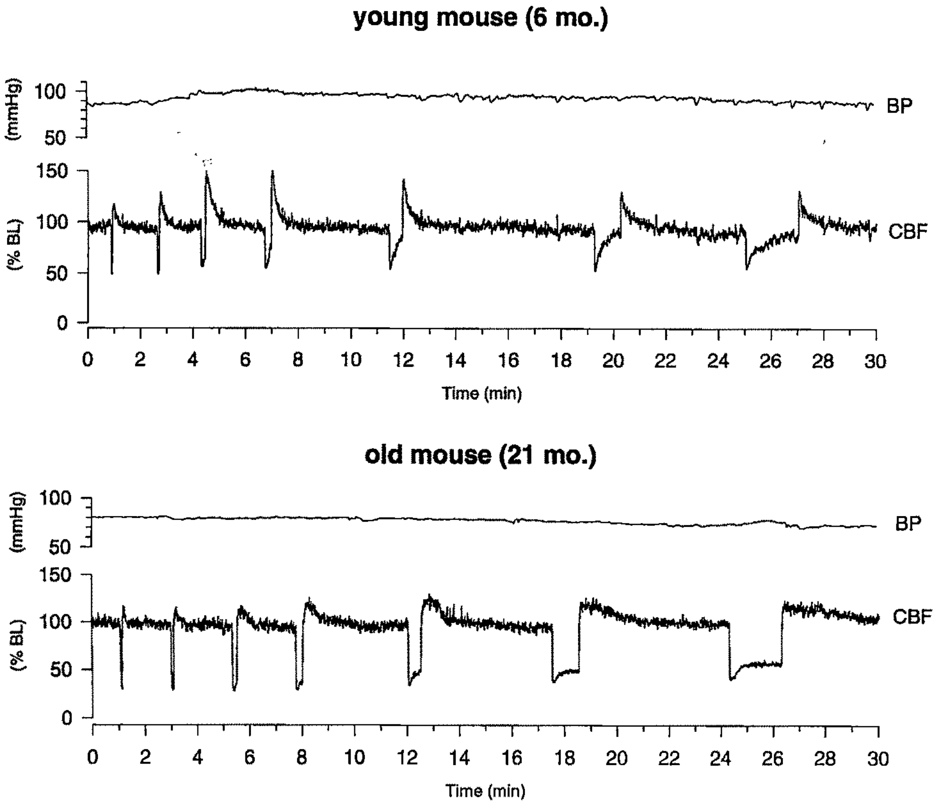

Fig 2. Response of blood pressure (BP) and CBF to 3-, 5-, 10-, 15-, 30-, 60-, and 120-second AICA clampings in young and aged mice. Abrupt decline in CBF is observed with clamp initiation, followed by gradual recovery toward BL during 30-, 60-, and 120-second occlusions in both animals. Decrease amplitude is larger and recovery amplitude is smaller in aged mouse.

so vascular conductance is defined as the flows BO and CO', respectively.

Thus, to quantify CBF autoregulation, the compensatory dilatory response (vascular conductance change) of the cochlear vessels during AICA occlusion was calculated as follows: Vascular responsiveness = CO'/BO.

Percent AICA contribution and vascular responsiveness were determined from the 60-second occlusion in each animal, because shorter occlusions did not permit for maximum response of the vessels (Fig 2).

Statistical Analysis. All data are reported as mean ± SD. Statistical significance of differences between the groups was evaluated by use of Student's *t*-test or the Mann-Whitney rank sum test.

RESULTS

Mean values for BP immediately before the first occlusion and arterial Po2, Pco2, and pH at the end of the experiments in young and aged groups are summarized in the Table. There was no significant difference between the two groups for these parameters. Indeed, these groups were surprisingly similar.

Figure 2 shows a series of BP and CBF responses to AICA clampings of different durations in a young mouse and an aged mouse. Although irregular BP fluctuations are observed in both animals, none of them are obviously related to AICA clamping. An abrupt decrease in CBF is observed with AICA clamping. During the 30-, 60-, and 120-second clampings, a gradual recovery toward BL is observed in CBF. In general, the initial decrease in CBF with the onset of clamping in young mice is less than that in aged mice, and the amplitude of recovery is smaller in aged mice. This is a typical observation in these subjects. Upon clamp release, a reperfusion hyperemia of CBF is observed in both animals. The CBF increase immediately after clamp release is slow and the peak is not sharp in the aged mouse compared to the young mouse. In the aged mouse, the time required for CBF recovery to BL seems to have a positive relationship to the clamping duration, and the recovery times after the 60- and 120-second clampings are obviously longer than those in the young

BLOOD PRESSURE, BLOOD PH, AND BLOOD GAS VALUES

Group	*Age*	*Initial Blood Pressure (mm Hg)*	*pH*	*Po2 (mm Hg)*	*Pco2 (mm Hg)*	*N*
Young mice	6 mo	79.4 ± 7.5	7.337 ± 0.044	99.4 ± 12.6	33.7 ± 5.7	10
Aged mice	21 mo	79.3 ± 16.2	7.300 ± 0.032	93.0 ± 14.4	40.3 ± 10.0	10

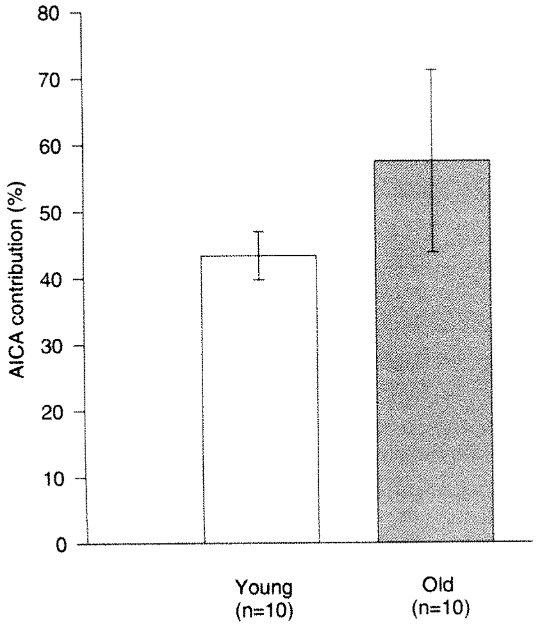

Fig 3. AICA contribution to CBF in young (n = 10) and aged (n = 10) groups. AICA contribution to CBF is significantly larger in aged group. Vertical bar — SD.

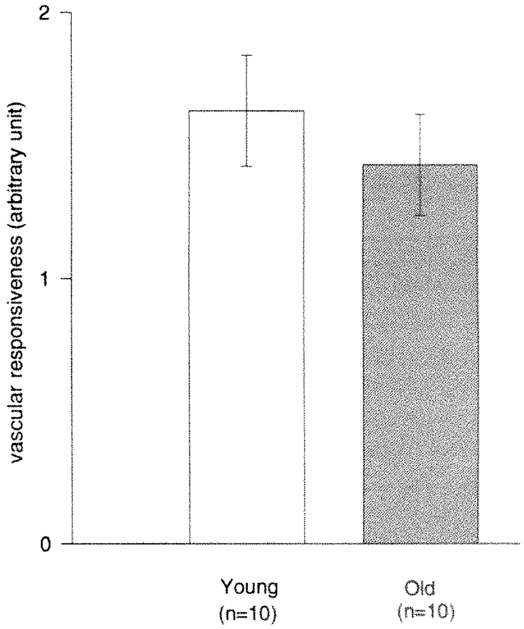

Fig 4. Vascular responsiveness in young (n = 10) and aged (n = 10) groups. Vascular responsiveness is significantly smaller in aged group. Vertical bar — SD.

mouse.

The mean values of percentage of AICA contribution to CBF were 43.4% ± 3.7% BL and 57.5% ± 13.7% BL for the young and aged groups, respectively (Fig 3). There was a statistically significant difference between the groups ($p < .05$; Mann-Whitney rank sum test used, since data failed the equal variance test). A greater within-group variation of AICA contribution was observed in the aged group.

Figure 4 illustrates the mean ± SD values of vascular responsiveness (change in vascular conductance) for the 60-second AICA clamping in both groups. The vascular responsiveness in the aged group (1.43 ± 0.19) was significantly lower than that in the young group (1.63 ± 0.21; $n = 20$, $t = 2.27$, $p < .05$).

To evaluate the potential relationship between the change in vascular conductance and the magnitude of the initial drop in CBF, we plotted the vascular responsiveness against the AICA contribution. Figure 5 shows this relationship. No significant correlation was found between them ($r = .368$, $p > .1$).

DISCUSSION

Several authors have reported CBF response to AICA occlusion in the course of developing a transient ischemia model without surgical injury to the auditory nerve and local pressure on the brain. Randolf et al[9] reported that compression of the cerebellar arteries in guinea pigs resulted in decreased CBF to 25% to 55% of the initial level in about half of their experiments and demonstrated great variation of collateral vessels in the cerebellar arteries. Seidman and Quirk[10] reported that the blood supply to the cochlea in the rat arises from multiple vessels and speculated that this anatomic feature could be a mechanism that provides stable blood flow and thereby organ homeostasis. Ren et al[8] demonstrated that the AICA contributes only about 45% of CBF in young adult guinea pigs; thus, 55% of CBF must come from other supplying vessels. They stated that

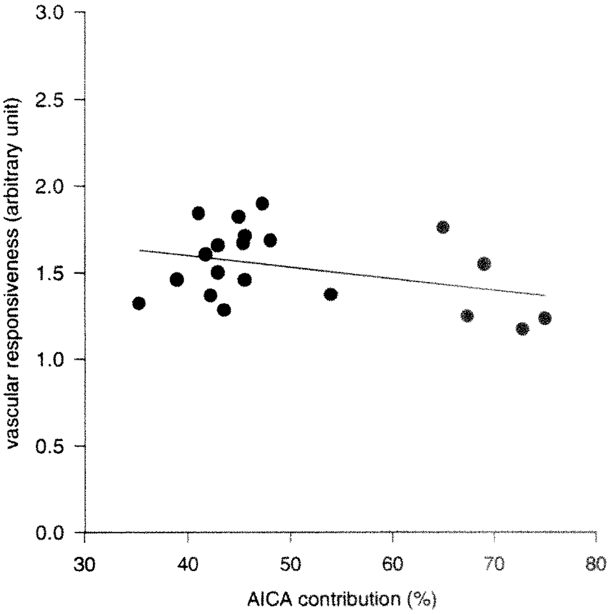

Fig 5. Relationship between AICA contribution and vascular responsiveness. No relationship existed between them ($y = 0.868 - 0.0067x$, $r = .368$, $p > .1$).

AICA clamping is not a suitable model for investigation of ischemia effects in the guinea pig cochlea, but that it is a useful approach for studying autoregulation of the cochlear vascular system.

In the present study, the mean value of AICA contribution to CBF in young mice was 43.4% ± 3.7%, which was similar to the value reported in young adult guinea pigs.[8] On the other hand, the AICA contribution in aged mice (57.5% ± 13.7%) was significantly higher than that in young mice. This finding indicates that the relative contribution to CBF from supplying vessels other than the AICA decreases with aging. A few studies have reported, in other organs, that with occlusion of the major supplying artery there is an effect of aging on collateral vascular function. Yamaguchi et al[11] demonstrated that collateral vascular function through pial anastomoses in the brain deteriorates with aging in cats. They speculated that decreased collateral function may be one of the factors that increases the vulnerability of the aged brain to ischemic insult. Our results indicate that collateral vascular function, the supply of blood to the cochlea from vessels other than the AICA, deteriorates with aging. Therefore, decreased collateral vascular function may be one of the factors that lead to impaired organ homeostasis in the aged cochlea.

In this study, using the AICA clamping model, autoregulation of CBF, as defined by the change in vascular conductance, was quantified in each animal. The advantage of using this model is that local perfusion pressure can be manipulated without systemic BP change, which may elicit neurohumoral effects. In the previous studies using this model,[7,12] the gain factor ($1 - \Delta F/\Delta P$) was calculated to quantify cochlear autoregulation, where ΔF is blood flow change and ΔP is perfusion pressure change during the clamping. In the present study, because the AICA contribution to CBF is significantly different between the young and the aged groups, reduction in cochlear perfusion pressure during the clamping (ΔP) is also significantly different between the groups. Because the gain factor ($1 - \Delta F/\Delta P$) is a ΔP-dependent measurement, it is inappropriate to compare gain factors for comparing the CBF autoregulation between the two groups.

It has been demonstrated that autoregulation of blood flow occurs mainly within individual arterioles[13]; therefore, resistances of the large supplying vessels can be considered to be relatively constant during clamping. In Fig 1, the perfusion pressure existing immediately after clamping (B) and that existing just before the clamp release (C) are assumed to be equal. Consequently, the ratio of blood flow at C to blood flow at B (segment CO' to segment BO) indicates the ratio of vascular conductance at C and B. Therefore, the vascular responsiveness calculated by CO'/BO reflects the dilation response of the cochlear vessels that compensates for reduced perfusion pressure during the clamping.

Although the AICA contribution to CBF is different between young and aged groups (Fig 3), no significant correlation exists between the AICA contribution and the vascular responsiveness (Fig 5). This observation eliminates the possibility that the vascular responsiveness difference between the groups was dependent on a differential AICA contribution. Our data (Fig 4) show that the vascular responsiveness in the aged group was significantly lower than that in the young group. This finding suggests that the cochlear vessels of the aged ear have less capacity to compensate for reduced perfusion pressure (autoregulation) compared to those of the young one.

In summary, the present study reveals that 1) the autoregulation of CBF is reduced by aging and 2) the collateral vascular function supplying blood to the cochlea deteriorates with aging. These two changes in vascular function suggest that the aged ear is more sensitive to stress factors that affect cochlear function.

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The 7th International Symposium on Recent Advances in Otitis Media will be held June 1-5, 1999, in Fort Lauderdale, Florida. For information, contact David J. Lim, MD, House Ear Institute, 2100 W Third St, Los Angeles, CA 90057.

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MANAGEMENT OF THE TINNITUS PATIENT

The Sixth Annual Conference on the Management of the Tinnitus Patient will be held in Iowa City, Iowa, September 18-19, 1998. Professionals who provide tinnitus management and patients who suffer from tinnitus are invited to attend. For information, please contact Richard Tyler, PhD, Director of Audiology, The University of Iowa Department of Otolaryngology–Head and Neck Surgery, 200 Hawkins Drive C21GH, Iowa City, IA 52242; telephone (319) 356-2471; fax (319) 353-6739; email rich-tyler@uiowa.edu.

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TRANSNASAL ENDOSCOPIC REMOVAL OF AN EXTENSIVE IMMATURE TERATOMA IN A THREE-MONTH-OLD CHILD

RICHARD LOUIS VOEGELS, MD
SÃO PAULO, BRAZIL
WOLFGANG LUXEMBERGER, MD
GRAZ, AUSTRIA
HEINZ STAMMBERGER, MD
GRAZ, AUSTRIA

The use of endoscopy in the paranasal sinuses has grown and diversified in the past few years. We present a case of an immature malignant teratoma originating from the sinuses, with intracranial extension to the middle and posterior fossae, that was managed and removed endoscopically. At the age of 2 months, the patient was hospitalized for respiratory instability. Diagnosis was made with biopsy, and chemotherapy was introduced. After 4 weeks, an endonasal endoscopic approach was performed, and a complete macroscopic removal of the tumor was achieved without complications. Follow-up for 10 months with magnetic resonance imaging and endoscopic examination has not revealed recurrence. In selected cases, the endonasal endoscopic technique may be a good alternative to the external approach, reducing the operative trauma and mortality.

KEY WORDS — endoscopic surgery, head and neck surgery, nasopharynx, teratoma, tumor.

INTRODUCTION

Teratomas originate from pluripotent cells that develop into neoplasms composed of a wide diversity of tissues foreign to the site from which they arise. They rarely occur in the head and neck[1-6] and may cause considerable morbidity or mortality secondary to respiratory obstruction. The treatment of choice is prompt surgical removal.

The purpose of this paper is to present a case of an immature undifferentiated teratoma originating from the sinuses, with intracranial extension to the middle and posterior fossae, that was managed and removed endoscopically. To our knowledge, it is the first case described in the literature of a teratoma removed with this technique.

CASE REPORT

A full-term girl with Dandy-Walker syndrome was born spontaneously. Computed tomography (CT) performed 2 days postpartum showed a cyst in the cerebellum confirming the diagnosis of Dandy-Walker syndrome. Further, an agenesis of the corpus callosum was noted. The teratoma, already present at this time, had not been diagnosed, probably because of the other disorders, and was misinterpreted by the radiologist as ectopic brain tissue.

At the age of 6 weeks, the child was referred to an otolaryngology specialist because of nasal obstruction and bloody secretion from the right nostril. Physical examination revealed a polypoid mass in the right nostril. A CT scan showed a soft tissue mass in the right nostril and ethmoid complex, possibly originating from the nasopharynx and/or the retromaxillary region (Fig 1). The lesion extended along the skull base lateral to the internal carotid artery, elevating dura of the middle cranial fossa and indenting dura of the posterior cranial fossa. The latter effect resulted in considerable compression of the brain stem.

The child was referred to our hospital and a biopsy specimen was taken endoscopically. Histology revealed an immature teratoma with 20% neuroectodermal tissue, grade 2, according to Gonzalez-Crussi.[1]

Meanwhile, the child required intensive care due to intermittent respiratory instability, most likely due to brain stem compression by the teratoma. Immediately after establishment of the diagnosis, two cycles of chemotherapy were introduced by the pediatric oncologists (Makei protocol 1989, consisting of etoposide, cisplatin, vincristine sulfate, and ifosfamide).

When the patient was 3 months old, after discussion with the neurosurgeons of the possible surgical approaches and respective risks, we performed an endonasal endoscopic approach to remove the tumor. A complete macroscopic removal of the tumor was achieved without complications. Five days postoperatively, the child was discharged from intensive care, and her respiratory function was normal. After

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From the Department of Otolaryngology, University of Graz Medical School, Graz, Austria.

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CORRESPONDENCE — Richard Louis Voegels, MD, Rua Iubatinga, 145 ap. 61-B, São Paulo, SP 05716-110 Brazil.

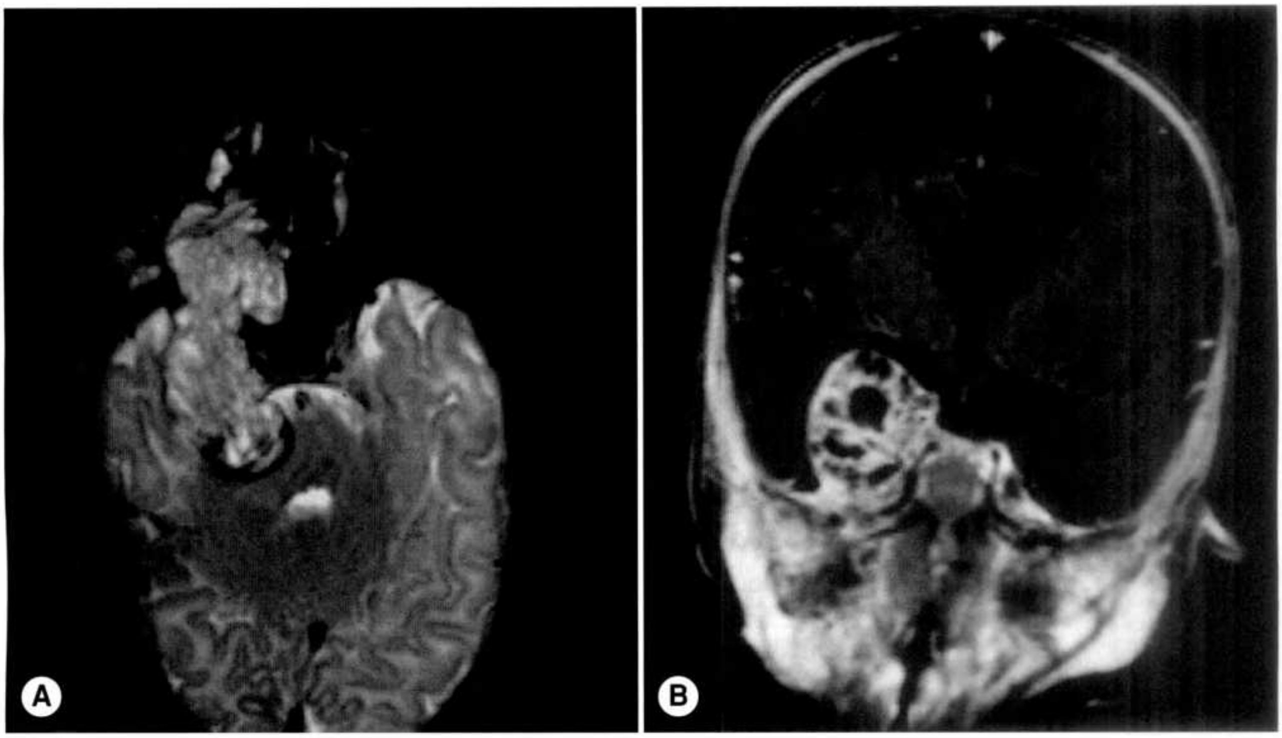

Fig 1. Computed tomograms. A) Axial view, showing soft tissue mass in right nostril and ethmoid complex, extending along skull base lateral to internal carotid artery. B) Coronal view, showing soft tissue mass elevating dura of middle cranial fossa.

that, one more course of chemotherapy following the Makei protocol was given. We did not then continue chemotherapy, proceeding on the assumption that the teratoma had become mature under the last cycles of chemotherapy, because histologic examination showed no more immature neuroectodermal tissue in the specimen, in contrast to the histology of the biopsy.

Follow-up for 10 months with magnetic resonance imaging and endoscopic examination has not revealed recurrence.

Histopathologic Findings. The specimen presented as a heterogeneous lesion with partially immature tissue structures, equivalent to descendants of the three germ layers. Many tubular structures (neuroectodermal tissue) could be identified, partially rich in pigmentation (incompletely differentiated retina; Fig 2A). There were also glandular structures with cylindric cells and goblet cells. Some were surrounded by primitive cartilaginous structures and/or irregular musculature (bronchial structures). Singular cells of the epithelium of these glandular structures could be identified as Paneth cells (intestinal epithelium; Fig 2B). Further, groups of polygonal epithelial cells with eosinophilic, granulated cytoplasm and nuclei rich in chromatin were reminiscent of liver cells.

Immunohistochemically, these cells reacted positively with antibodies against pancytokeratin and alpha-fetoprotein. More than 20% of the biopsy material was immature neuroectodermal tissue, so the lesion was staged grade 2 according to Gonzalez-Crussi.[1]

Surgical Approach. Under general anesthesia, the nasal mucosa was decongested with cottonoids soaked in Adrenalin 1:1,000. With a 0°, 2.7-mm rigid telescope (Karl Storz, Tuttlingen, Germany), the lesion was approached through the right nostril and first removed from the nasal cavity. The middle turbinate, apart from rudiments, was nonexistent, apparently due to the pressure of the lesion. There was no adhesion or infiltration toward the septum, nasal floor, or anterior skull base. The lesion reached the dura of the anterior skull base in the posterior ethmoid region, but could be well separated from the latter. The bulk of the anterior portion of the lesion reached behind and lateral to the maxilla, where it was more firmly attached; however, it did not show signs of muscle infiltration and could be removed with what appeared to be capsular tissue. The lesion was of contrasting consistencies: soft, almost polypoid areas alternated with almost cartilaginous portions. Due to its size, the lesion had to be removed in a piecemeal fashion with suitable forceps. One finger reached into the (nonpneumatized) sphenoid, and another through the choana into the nasopharynx. These portions were removed without any difficulty. Then, the dura of the middle cranial fossa was exposed and the lesion was carefully dissected.

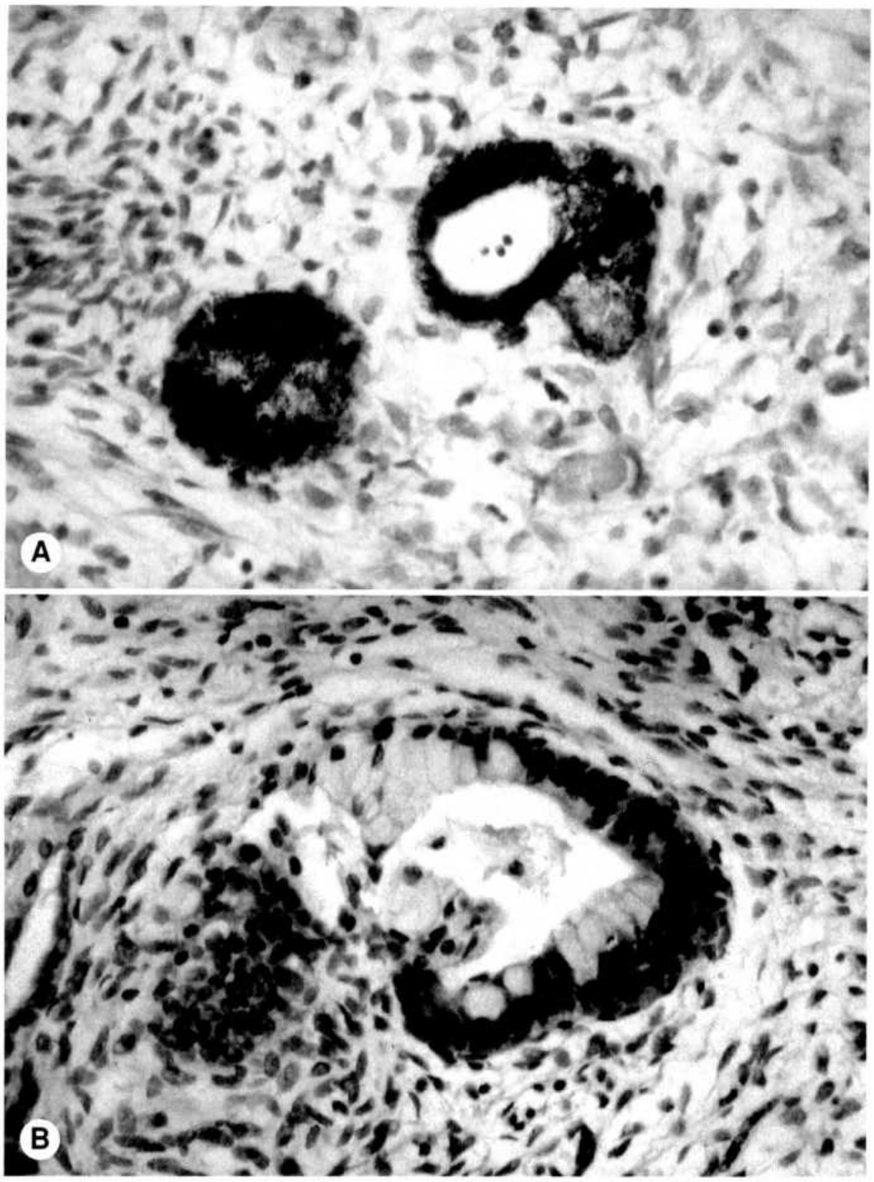

Fig 2. Histopathologic findings. A) Plenty of tubular structures (neuroectodermal tissue), partially rich in pigmentation (incompletely differentiated retina). B) Glandular structures with cylindric cells and goblet cells. Some of them are surrounded by primitive cartilaginous structures and/or irregular musculature (bronchial structures). Singular cells of epithelium of these glandular structures can be identified as Paneth cells (intestinal epithelium).

Medially, the anterior course of the internal carotid artery was exposed from the carotid foramen up to the cavernous sinus; the artery was only covered by thin fibers that appeared tissular. Finally, we succeeded in removing the anterior lesion, dissecting it off the posterior cranial fossa dura. No significant bleeding was encountered during the entire procedure; the blood loss was less than 15 mL. The cavity was loosely packed with resorbable gauze (Sorbacel), and no other packing was used. The entire procedure was performed with a neurosurgeon in the operating room on standby, should any infiltration of the dura have been present or other problems encountered.

DISCUSSION

Grass, in 1691, described the first teratoma of the neck. The first microscopic description of teratoma was given by Hess in 1854.[4] In most series, the incidence of teratomas of the head and neck comprise only 3.5% or less of all cases.[5,7] The differential diagnosis of a nasopharyngeal teratoma in an infant includes: chordomas, encephaloceles, craniopharyngiomas, hamartomas, rhabdomyomas, and gliomas.[8,9] Delay in diagnosis may result in airway obstruction and death. Prompt intervention with surgical removal of the tumor is required. The operative mortality has been reported to be between 9.7% and 17%.[10,11]

The use of endoscopy in the paranasal sinuses probably dates to 1901, when Hirschman used a modified cystoscope to examine the sinuses,[12-14] but it was only in 1978 that Messerklinger[15] and Wigand et al[16] introduced the concept of sinus surgery with an endoscope. Since then, the use of an endoscope during surgery has increased and diversified.[17-19]

In our patient, the tumor originated in the nasopharynx and extended to the posterior fossa. We used the endoscope to access and remove this tumor, avoiding major surgical trauma. Bleeding was minimal, and the child had a fast and satisfactory recovery. Follow-up of 10 months with magnetic resonance imaging has shown no signs of recurrence.

CONCLUSION

Teratomas of the nasopharynx are rare, but require prompt diagnosis and intervention. The treatment of choice is surgical removal, normally associated with a high rate of operative mortality. In this case, the endoscopic approach was effective in removing the lesion, as preoperatively, there was a high degree of probability that the lesion had not distracted the dura, despite its gross placement in the middle and posterior fossae. Considering the potential risks of a transcranial approach to these difficult regions, we agreed with the neurosurgeons to attempt the approach described in this paper. Thus, major surgical trauma and its sequelae could be avoided. We believe that in well-selected cases this technique may be a good alternative to an external or even a transcranial approach, reducing the operative trauma and mortality. If the endoscopic technique had resulted in an incomplete debulking of the lesion, other approaches could have been applied at a later stage.

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CONSERVATIVE APPROACH TO INFLAMMATORY NASOFRONTAL DUCT DISEASE

JOSEPH B. JACOBS, MD
VICTOR M. LAGMAY, MD
RICHARD A. LEBOWITZ, MD
ANGELA DAMIANO, MD

NEW YORK, NEW YORK

Endoscopic data reveal that mucosal disease frequently recurs following the surgical treatment of chronic diffuse hyperplastic frontal sinus and nasofrontal duct disease. However, this group of patients often remains asymptomatic. We present 40 patients who underwent endoscopic sinus surgery with a 4-year mean follow-up. Postoperative endoscopy revealed mucosal disease within the frontal recess in 39 of the 40 (97.5%) patients; however, only 3 of the 40 (7.5%) were symptomatic. Of these 3 patients, 1 underwent revision surgery. Historical information demonstrates the potential for significant nasofrontal duct stenosis associated with such revision surgery. On the basis of our initial results, endoscopic observation and medical therapy seem to be successful in controlling the symptoms associated with this disease process.

KEY WORDS — endoscopic sinus surgery, frontal sinusotomy, nasofrontal duct disease.

INTRODUCTION

Chronic inflammatory hyperplastic sinus disease is a medical condition associated with different causes. Surgery, in combination with medical therapy, may be undertaken to relieve paranasal sinus outflow tract obstruction and remove bulky airway disease. However, numerous reports utilizing postoperative endoscopy clearly delineate a significant tendency for recurrence of mucosal disease within these outflow tracts.[1-4] In view of this natural history of chronic sinusitis, the additional surgical risk of frontal sinusotomy, and the potential for secondary nasofrontal duct (NFD) stenosis, we must critically evaluate therapy for recurrent NFD disease.

Over the last century there has been an ongoing debate in the literature regarding surgical approaches for chronic frontoethmoid sinusitis.[2] The pendulum has swung between various intranasal and external techniques. Except in the case of obliteration, all of these procedures, whether conventional intranasal, endoscopic, or external, require the removal of obstructing mucosal disease and maintenance of NFD function for success. In addition, direct external surgery of the frontoethmoid complex often requires some type of duct reconstruction.

Published results demonstrating variable success with these techniques attest to the difficulty associated with the requirements of removal of disease and preservation of adequate NFD bony architecture and mucosa while providing permanent drainage and ventilation.[5-7] Further, evidence from animal studies, as well as clinical observation, suggests that mucosal stripping and bone removal within the varied and narrow confines of the NFD create a significant inflammatory response.[5,8-11] Subsequent irregular regeneration of mucosa and bone may precipitate symptomatic outflow tract stenosis.

The concept of functional endoscopic sinus surgery emphasizes the removal of disease within the ostiomeatal complex while relieving outflow tract obstruction and preserving normal mucosa. Substantial clinical evidence exists demonstrating the success of this approach for maxillary antrostomies with an associated lack of complications.[1] However, according to the historical surgical perspective for frontal sinus surgery, as well as the natural history of inflammatory sinus disease, otolaryngologists must carefully balance the relative benefits of this concept for frontal sinus outflow tract surgery.

MATERIALS AND METHODS

The records of 110 patients who underwent endoscopic frontoethmoid surgery for chronic inflammatory sinusitis by the senior author (J.B.J.) between July 1991 and November 1993 were reviewed. Patients with no history of previous sinus surgery with diffuse hyperplastic or polypoid sinus disease, Kennedy stage III or IV,[1] and with a minimum 3-year follow-up were included in the analysis. Thus, the study group was based on 40 patients who underwent 61 such procedures.

During the preoperative evaluation, the patient's

From the Department of Otolaryngology, The New York University School of Medicine, New York, New York.

CORRESPONDENCE — Joseph B. Jacobs, MD, Dept of Otolaryngology, The New York University School of Medicine, 550 First Ave, New York, NY 10016.

POSTOPERATIVE ENDOSCOPIC FINDINGS

	No.	%
Mucosal hypertrophy	28	45.9
Recurrent polyps	30	49.2
Discharge	22	36.1
Inflammation	12	19.7
Adhesions	4	6.6
Crusting	3	4.9
Stenosis	1	1.6

n = 61 operated sides.

history was reviewed and relevant medical information, as well as the findings of computed tomography (CT) scans and endoscopy, were recorded. Surgery was performed on patients who failed medical therapy and manifested persistent and significant symptoms.

A modified Messerklinger approach was employed, usually under general endotracheal anesthesia. The extent of surgery was determined by findings on preoperative endoscopy and CT scan, as well as by the extent of mucosal disease and anatomic variations noted during the surgery. A conservative dissection was performed within the frontal recess. Polyps and hyperplastic mucosa were cleared; however, inflammatory tissue, often noted to be associated with increased bleeding, was not stripped. Direct visualization of the frontal ostium was not always obtained. Persistent inflammatory mucosa within the frontal sinus outflow tract was treated medically in the postoperative period.

During postoperative endoscopy, the appearance of the mucosa in the surgical bed, including evidence of hypertrophy or polyps, discharge, inflammation, adhesions, crusting, or stenosis, was noted. At the final visit within the parameters of the study, the endoscopic findings within the frontal sinus outflow tract, as well as patients' reported improvement in symptoms, were recorded.

RESULTS

Patient Characteristics. Patients ranged in age from 16 to 69 years. The most frequent preoperative symptoms were nasal obstruction (100.0%), nasal discharge (100.0%), change in olfaction (62.5%), infection (35.0%), and headaches or facial pain (17.5%).

Thirteen patients had a history of asthma, 3 were sensitive to aspirin, and 2 had Samter's triad. Of the 40 patients, 37 were referred or had been evaluated for allergy. Of these 37, 26 had positive skin tests and 19 had been or were currently undergoing desensitization.

Preoperative Radiographic Findings. Evidence of frontal sinus and/or frontal recess mucoperiosteal disease was found on 61 sides of the 40 patients in the study group. All patients had preoperative CT scans in the coronal plane, 35 had axial scans, and 28 had sagittal reformats of the frontal sinus outflow tract.

Preoperative Endoscopic Findings. There was bilateral diffuse polypoid sinus disease in 17 patients, while 12 presented with middle meatal polyposis and 11 with hyperplastic mucosa.

Surgical Procedures. Of the 40 patients, 21 had bilateral frontal sinusotomies and 19 had unilateral ones, for a total of 61 frontal sinus procedures.

Complications. No major complications were encountered in the study group. Two patients developed brief medial periorbital ecchymosis, 1 of whom was noted to have periorbital fat exposure during the surgery.

Postoperative Endoscopic Findings. By our endoscopic criteria, 39 of the 40 (97.5%) patients were noted to have mucosal disease within at least one frontal sinus outflow tract. Of the 61 surgical sites, 58 (95.1%) were noted to be abnormal by endoscopy (see Table). The frontal ostium was not clearly visualized by endoscopy in 50 (82.0%) of the 61 operated sides.

Postoperative Symptomatology. The follow-up period was 37 to 60 months, with a mean of 48 months. Overall, 37 of the 40 (92.5%) patients reported an improvement in symptoms at the final visit within the parameters of the study. The remaining 3 noted little or no improvement, and 1 of these required additional surgery due to relative frontal sinus outflow tract stenosis. No patients felt they were worse following surgery. For the 37 improved patients, medical therapy continued to be prescribed as needed.

DISCUSSION

The evaluation of the results of paranasal sinus surgery is difficult, since there are no generally agreed-upon objective criteria. In addition, an apparent dichotomy exists between subjective improvement following surgery and both endoscopic findings of outflow tract patency and evidence of synchronous mucosal disease within operative sites.[1-3,12] Moreover, potential clinical implications may vary for each region.

The frontal sinus outflow tract is a complex, anatomically variable mucosal-lined bony pathway between the frontal sinus and the anterior ethmoid region from which it originates.[13] The postoperative evaluation of frontal sinus outflow tract patency appears to be a more complex issue than that of tract patency in the maxillary and ethmoid regions.

Kennedy[1] reported on results of endoscopic sinus surgery in 120 patients followed for a mean of 18 months. He noted overall maxillary antrostomy patency to be 97.3% in 227 endoscopic procedures, although 44.9% of the postoperative cavities contained residual inflammatory mucosal disease. In 34 patients with diffuse polyposis who underwent 68 procedures, antrostomy patency was 92.6%, while frontal recess stenosis occurred in 27.3%. Of these 68 operative sites, 76.5% of the cavities had evidence of residual mucosal disease. Of the 120 patients in the study group, 97.5% reported an improvement of symptoms, and of 34 patients with diffuse polyposis, 97.1% were improved.

The series of Wigand and Hosemann[12] that evaluated objective findings and subjective results of endoscopic frontal sinus surgeries is the most comprehensive in the literature. They noted that of 162 frontal sinusotomies with an average follow-up of 3.5 years, 60.0% were distinctly abnormal by endoscopy. In addition, of these 162 operative sites, 30.0% could not be demonstrated to be patent by endoscopic probing. However, they stated that the majority of these patients were asymptomatic and only 5.0% required revision surgery. They therefore concluded that endoscopic patency of the NFD is not a requirement for adequate mucociliary clearance.

Hosemann et al[4] reported on the results of 154 frontal sinusotomies in 88 patients followed for a mean of 13 months. Of these 154 sites, 91 (59.1%) were patent by probing, only 34 (22.1%) were visible by endoscopy, and 29 (18.8%) were inaccessible both by probing and by endoscopic visualization. Of the 88 patients who underwent these procedures, 87.0% were improved following surgery.

Jacobs[2] evaluated postoperative endoscopic findings within the frontal sinus outflow tract. In patients with diffuse polyposis, 46 of 55 (83.6%) sites were abnormal, compared with 15 of 26 (57.7%) in patients with middle meatal polyps and 13 of 24 (54.2%) in patients with hyperplastic sinusitis. The mean follow-up was 16 months. Improvement of symptoms was noted by 83.4%, 85.7%, and 86.7% of these patients, respectively.

The reported results of postoperative endoscopy and improvement of symptoms from the above studies are similar to our present findings. By endoscopy, 39 of our 40 (97.5%) patients with diffuse hyperplastic or polypoid sinus disease were noted to have at least 1 abnormal postoperative NFD site, for a total of 58 (95.1%) of 61 frontal sinusotomies. However, 37 of the 40 (92.5%) patients reported an improvement in symptoms with a mean 4-year follow-up.

The cause of chronic inflammatory sinusitis is clearly multifactorial, and mucosal hypersensitivity will remain a constant dilemma following surgical resection of diseased and obstructing mucosa within the ostiomeatal complex. The frontal sinus outflow tract appears to be preferentially involved with recurrent mucosal disease and associated lack of endoscopic patency as compared to maxillary antrostomies. However, the fact that subjective symptoms remain controlled indicates adequate mucociliary clearance. According to the objective findings of mucosal disease, therefore, postoperative endoscopy provides an unparalleled opportunity to guide medical management and control symptoms.

CONCLUSION

Postoperative endoscopy provides data concerning outflow tract patency and the presence of inflammatory mucosal disease within the ostiomeatal complex. There is a significant incidence of recurrent mucosal disease within the NFD compared to the maxillary outflow tract. However, the fact that with appropriate medical therapy these patients remain asymptomatic suggests the presence of adequate mucociliary function. In view of the unique anatomy of the frontal sinus outflow tract, the potential for symptomatic stenosis following revision surgery, and the natural history of chronic sinusitis, postoperative endoscopy provides a desirable opportunity to conservatively manage patients with persistent NFD mucosal disease.

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RHINOLOGY SYMPOSIA

The 18th International Symposium on Infection and Allergy of the Nose (ISIAN), the 4th Asian Research Symposium on Rhinology (ARSR), and the 3rd ASEAN Rhinology Group Congress (ARC) will be held in Manila, Philippines, February 14-18, 1999. For information, contact Gil M. Vicente, MD, Congress President, Dept.of ORL-HNS, Jose Reyes Memorial Medical Center, Rizal Avenue Sta. Cruz, Manila, Philippines 1003; telephone (632) 743-6921; fax (632) 732-1077 or (632) 522-0946; e-mail isian@psohns.com; web site http://www.psohns.com/isian.

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EXAMINING THE SAFETY OF NASOGASTRIC TUBE PLACEMENT AFTER ENDOSCOPIC SINUS SURGERY

NEIL BHATTACHARYYA, MD HARSHA V. GOPAL, MD
BOSTON, MASSACHUSETTS

Several cases of intracranial injury during the placement of nasogastric tubes have been reported, usually in the setting of anterior skull base fractures. The fovea ethmoidalis and sphenoid sinus are often exposed after endoscopic sinus surgery, so that these structures are potentially placed in the line of contact during nasogastric tube placement. In order to evaluate the ability of the fovea ethmoidalis and roof of the sphenoid sinus to withstand penetration from possible contact during nasogastric tube placement, 12 fresh cadaver heads were studied. After complete endoscopic ethmoidectomy and wide sphenoidotomy, standard 18F and 16F nasogastric tubes were inserted to produce deliberate direct contact with both the fovea ethmoidalis and the sphenoid sinus roof. No penetrations of the fovea occurred in 20 specimen sides with the 18F tube; penetration did occur with the 16F tube in 1 of 13 sides (7.7%). With respect to the sphenoid sinus, no intracranial penetrations occurred in 16 and 11 sides for the 18F and 16F tubes, respectively. The sphenoid sinus was easily entered even in the presence of an intact middle turbinate. These data suggest that although intracranial penetration during nasogastric intubation after endoscopic sinus surgery is an unlikely event, there is a non-negligible risk of such injury. Nasogastric intubation should be performed with caution in patients with a history of sinus surgery.

KEY WORDS — complications, endoscopic sinus surgery, nasogastric tube.

INTRODUCTION

Endoscopic sinus surgery (ESS) is becoming increasingly common within the US population as chronic sinusitis becomes better understood as a significant cause of patient morbidity and economic cost.[1] In addition, nasogastric tube (NGT) placement is an extremely common procedure. Though no cases of anterior skull base penetration due to NGT placement after ESS have been reported, as the number of patients undergoing sinus surgery increases, a potential interaction with NGT placement is likely to arise. Since the bone of the anterior skull base is often exposed after ESS, NGT placement may potentially injure or penetrate the anterior skull base. This study was undertaken to determine if exposure of the anterior skull base after ESS renders it more susceptible to injury during NGT placement.

METHODS

Twelve fresh frozen cadaver heads were studied. After allowing the specimen to thaw, ESS was performed with standard technique. This consisted of bilateral anterior and posterior ethmoidectomies, middle meatal antrostomies, and wide sphenoidotomies performed by several practicing otolaryngologists. In all cases, the middle turbinate was infractured, and middle turbinectomies were performed in approximately 50% of the sides.

After the dissections were completed, the nasal cavities of each cadaver head underwent serial examination. Each side of each specimen was examined with both endoscopic visualization and palpation for natural and surgically created dehiscences in the ethmoid and sphenoid sinus roofs, and the presence or absence of the middle turbinate. Sides with significant (greater than 3 mm) dehiscences were then excluded. Under endoscopic guidance, standard 18F and 16F NGTs were inserted into the nasal cavity with intentional traumatic contact with the fovea ethmoidalis, working from posterior to anterior with multiple impacts. Observation was made as to whether the tube penetrated the ethmoid roof or whether the tube kinked rather than penetrating the bone. The tube was then inserted into the sphenoid sinus and the same observation was made. Also, the ease with which the NGT entered the sphenoid sinus versus entering the choana was noted. Whether the middle turbinate, if present, offered significant protection for either the ethmoid roof or the sphenoid sinus was assessed. If the turbinate prevented the tube from contacting the roof or entering the sphenoid sinus, the turbinate was displaced medially and the insertion was continued.

RESULTS

Twelve fresh cadaver heads initially provided 24 dissected sides. With respect to the ethmoid sinus, 4 sides were excluded because of large dehiscences of the fovea ethmoidalis. Two specimen sides had very small dehiscences approximating 3 mm, much smaller than the NGT diameter, and were included

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From the Joint Center for Otolaryngology and the Department of Otology and Laryngology, Harvard Medical School, Boston, Massachusetts.
CORRESPONDENCE — Neil Bhattacharyya, MD, Joint Center for Otolaryngology, 333 Longwood Ave, Boston, MA 02115.

SUMMARY OF DATA

Nasogastric Tube	*Sinus*	*No. of Sides**	*Presence of Middle Turbinate*		*Penetrations*	
			No.	*%*	*No.*	*%*
18F	Ethmoid	20	9	45.0	0	0
	Sphenoid	16	8	50.0	0	0
16F	Ethmoid	13	5	38.5	1	7.7
	Sphenoid	11	5	45.5	0	0

*Sides with dehiscences >3 mm were excluded.

in the subsequent evaluation. The middle turbinate was present in 9 of the 20 (45%) sides studied. The deliberate contact between the 18F nasogastric tube and the fovea resulted in no penetrations in 20 sides. Specifically, the 2 sides with small dehiscences did not allow penetration of the fovea. The presence or absence of the middle turbinate did not preclude contact between the NGT and the fovea. Thirteen sides were studied with the 16F NGT. One of these sides had a 3-mm dehiscence. Deliberate contact with the 16F tube resulted in 1 fovea penetration (7.7%). This violation occurred lateral to the cribriform plate between the anterior and posterior ethmoid arteries in the absence of the middle turbinate, and was not in the side with the 3-mm dehiscence. The middle turbinate was present in 5 of the 13 sides (38.5%). The presence or absence of the middle turbinate did not preclude the contact between the NGT and the fovea.

The NGT entered the sphenoid sinus easily in 22 of 24 (91.7%) specimen sides. The middle turbinate prevented easy entry into the sphenoid sinus in the 2 remaining cases. In 2 cases, the NGT preferentially entered the sphenoid sinus rather than the choana because of prominent inferior septal spurs. There were 9 specimen sides with native or postdissection dehiscences in the roof of the sphenoid sinus; in 1 of these the dehiscence was 3 mm, and it remained included. The remaining 8 sides were excluded from further study. The 18F nasogastric tube was unable to penetrate the roof of the sphenoid sinus in the 16 sphenoid sinus sides tested. Penetration also did not occur in the 11 sides tested with the 16F tube, including the side with the 3-mm dehiscence. These data are summarized in the Table.

DISCUSSION

Chronic sinusitis has been identified as a major cause of morbidity and economic cost in the United States.[2] The advent of ESS has increased the number of patients whose disease can be addressed relatively safely and effectively with decreased surgical morbidity. As more patients undergo ESS, there will be an increasing likelihood that patients undergoing NGT placement will have had ESS. These are the patients who may be at risk for complications.

Endoscopic sinus surgery affects structures other than the ostiomeatal complex alone. The effects of ESS on the physiology, stability, and growth of the nose have been well studied.[3,4] Others have addressed the risks of nasal osteotomies after ethmoidectomy, and found that osteotomies were relatively safe after ethmoidectomy.[5] Because of the degree of exposure of the ethmoid roof, and the possibility of a wide sphenoidotomy after ESS, this study was undertaken to determine if NGT placement imparts a greater risk in these patients. Placement of NGTs is one of the most common procedures in the hospital setting. Nasogastric tubes may be placed for gastric decompression, after bowel or head and neck surgery, for enteral tube feedings, or for a variety of other reasons. Though a case of intracranial penetration after sinus surgery has not been reported, several reports of intracranial placement have been noted.[6-8] Common to all of these reports is the presence of a fractured or weakened anterior skull base. One case of intracranial NGT penetration after transnasal transsphenoidal resection of a pituitary tumor has been reported.[9] The potentially serious consequences of severe brain injury and death likely from intracranial placement are well known, though surprisingly, the NGT may be removed with minimal sequelae.[8,10]

Overall, these results indicate that both the ethmoid and sphenoid sinus roofs are generally resistant to penetration by an NGT. In almost all cases, the NGT coiled in the nasal cavity rather than entering the intracranial space. Penetration intracranially requires two events. First, the NGT must contact the ethmoid roof or enter the sphenoid sinus. Second, the force generated at contact must be greater than the resilience of the skull base. In the case of the NGT, the force required to penetrate the skull base must be less than the force that will cause the tube to coil or kink. These data indicate that most often, the tube will coil before it can penetrate the skull base.

Of interest, in the cases that had very small dehiscences of the ethmoid or sphenoid sinus roof, the NGT was still not able to penetrate intracranially, despite the weakness created by the dehiscence. This supports the argument that the fovea ethmoidalis is indeed very strong bone. Despite the inherent strength of the anterior skull base, in 1 case, the NGT penetrated an otherwise intact fovea. In general, the fovea ethmoidalis may be thinned by surgery or extensive sinus disease, or may be naturally dehiscent, as in patients with spontaneous cerebrospinal fluid leaks.[11] These areas of thin or absent bone make penetration by an NGT more likely. The 18F NGT seemed to be safer than the 16F tube. This is likely due to the fact that the pressure exerted at the tip of the 16F tube is greater than that of an 18F tube. A larger tube appears safer in this situation.

In clinical situations, the risk to the anterior skull base from NGT placement is likely to be less than the risk assessed by this study. First, in awake and responsive patients, severe pain is likely to be encountered immediately when the NGT contacts the fovea, similar to the helpful signals of pain during ESS under local anesthesia. However, such warning signs are absent in anesthetized or unconscious patients. In addition, the middle turbinate probably does provide additional protection after healing has occurred. The turbinate does attain more rigidity than was evident in these cadavers, and it is also a very sensitive site when instrumented. Finally, the standard sphenoidotomy is not large enough for an NGT to enter the sinus. The risk to the sphenoid sinus roof is more applicable in cases of wide-field sphenoidotomies. Of interest, the specimens studied also illustrated the tendency for inferior septal spurs to increase the chance that the NGT would be aimed at the sphenoid sinus; this should be considered when selecting a side for NGT placement. Nevertheless, the data accumulated are for a "worst-case scenario," and should be considered when attempting NGT placement after ESS.

Though we cannot assess the overall risk of intracranial penetration due to small sample size, these results indicate that despite the overall resistance of the exposed anterior skull base to penetration, patients with previous ESS are at somewhat increased risk for intracranial injury during NGT placement. The placement of an NGT in these cases should be done carefully, and immediately terminated at the first sign of resistance or coiling of the NGT. Such cases of difficult placement could be done more safely under fluoroscopy or endoscopic guidance. We do not recommend methods that increase the stiffness of the NGT such as "icing" the tube prior to placement. Given the ease with which the sphenoid sinus was cannulated, with or without the presence of a middle turbinate, the NGT must be directed inferiorly in the nose, parallel to the hard palate and nasal floor. Aiming inferiorly alone may not be completely reliable, since in several cases, inferior septal spurs inadvertently directed the NGT toward the sphenoid sinus. We also recommend a larger tube, since the larger tube disperses the pressure of insertion over a wider area, and the larger tube is less likely to cannulate the sphenoid sinus.

CONCLUSIONS

1. Despite exposure after ESS, the fovea ethmoidalis remains generally resistant to penetration during NGT placement.
2. There is, however, a small increased risk of intracranial penetration after ESS, especially with the placement of smaller-gauge NGTs. A larger-bore NGT should be used in this setting.
3. Though a wide-field sphenoidotomy may allow the NGT to easily enter the sphenoid sinus, the sphenoid sinus roof is resistant to penetration by an NGT.
4. An NGT should be placed with caution after ESS. In patients in whom the tube coils in the nose or meets resistance, placement with endoscopic guidance should be considered.

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CYTOKINE GENE EXPRESSION IN NASAL POLYPS

CHUL HEE LEE, MD

CHAE-SEO RHEE, MD YANG-GI MIN, MD

SEOUL, KOREA

Nasal polyps are the most common mass lesion of the nasal cavity. Various pathogenetic mechanisms have been proposed. However, the cause is still largely unknown, and treatment methods have not been changed for several hundred years. In order to investigate the role of cytokines in the pathogenesis of nasal polyps, expression of cytokine messenger RNAs (mRNAs) in nasal polyps was investigated. We performed reverse transcriptase–polymerase chain reaction and Southern blot to examine gene expression of the cytokines interleukin (IL)–1ß, IL-6, IL-8, transforming growth factor (TGF)–ß, IL-4, IL-5, and interferon (IFN)–γ and compared the results with the gene expressions of these cytokines in normal nasal mucosa. Nasal polyp tissues were obtained from 14 patients undergoing polypectomy for nasal obstruction. Among them, 4 patients suffered from associated perennial allergic rhinitis. The mRNAs of IL-4 and IL-5 (Th2 cytokines) as well as IFN-γ (Th1 cytokine) were expressed in all of the nasal polyps obtained from the 14 patients, irrespective of the presence or absence of allergy, while 2, 0, and 4 of 6 normal turbinate mucosae expressed IL-4, IL-5, and IFN-γ mRNAs, respectively. The mRNAs of IL-1ß, IL-6, IL-8, and TGF-ß were expressed in 6, 1, 2, and 3 of 6 normal turbinate mucosae, respectively, while the mRNAs of these cytokines were expressed in all of the 14 polyp tissues except IL-6 mRNA, which was expressed in 13 nasal polyp tissues. There were no differences in the mean density ratios of each cytokine band on Southern blot between polyp tissues with allergy and those without allergy. These results suggest that many cytokines are produced in nasal polyps, that they may play important roles in the pathogenesis of nasal polyps, and that allergy per se may not play a fundamental role in the pathogenesis of nasal polyps.

KEY WORDS — allergy, cytokines, nasal polyp.

INTRODUCTION

Human beings have suffered for several thousand years from nasal polyps, which are the most common mass lesions encountered in the nose.[1] In spite of the frequency of their occurrence and long history of recognition, the cause and pathogenesis of nasal polyps are still largely unknown. Various pathogenetic mechanisms have been proposed by a number of investigators. Among them, two main theories — allergic and infectious — have been most frequently advocated. The major supporting features that favor the allergy theory are elevated histamine and immunoglobulin (Ig) E levels in extracellular polyp fluid,[2] and an increased number of IgE-bearing cells in nasal polyps of atopic patients.[3] On the other hand, several studies have cast doubt on the role of allergy as a cause of nasal polyps. The lymphocyte subpopulations,[4] the levels of antigen-specific IgE,[4] and the numbers of epithelial mast cells[5] in nasal polyps are not different between allergic and nonallergic groups. Other proposed pathogenetic mechanisms of nasal polyps include the infection theory, in which Norlander et al[6] proposed that infection induces mucosal trauma or epithelial desquamation that may be essential in initiating polyp formation. Dawes et al,[7] however, found no correlation between mucosal disease and bacteriologic findings from antral lavages. Another study comparing the distribution of cell types in polyps with that in normal nasal mucosa supports the theory that the pathogenesis of nasal polyps is associated with chronic inflammation and T-cell–dependent disturbances in the mucosa.[8]

During recent years, much attention has been directed to the roles of cytokines in the various inflammatory processes. It has been well recognized that an inflammatory process may be initiated by interleukin (IL)–1, IL-6, and IL-8. Transforming growth factor–ß (TGF-ß) may contribute to chronic inflammation and is associated with stromal fibrosis and thickening of the basement membrane. Numerous in vitro studies have suggested that many cytokines could play important roles in the pathogenesis of allergic inflammatory diseases.[9-11] Interleukin-4 is required for the production of IgE by inducing an isotype switch in B cells that is inhibited by interferon-γ (IFN-γ).[11,12] The major action of IL-5 is to stimulate the growth and differentiation of eosinophils and to increase their survival.[13,14]

In order to better understand the pathogenesis of nasal polyps, we investigated expression of various cytokine messenger RNAs (mRNAs) in nasal polyp tissues and compared it with that in normal nasal mucosa. To determine the role of allergy in polyp

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From the Department of Otorhinolaryngology–Head and Neck Surgery, Seoul National University College of Medicine, Seoul, Korea.

CORRESPONDENCE — Chul Hee Lee, MD, Dept of Otorhinolaryngology–Head and Neck Surgery, Seoul National University Hospital, 28 Yongon-dong Chongno-gu, Seoul, Korea.

formation, expression of cytokine genes known to play important roles in allergic inflammation was included in this study. For this purpose, we used a reverse transcriptase–polymerase chain reaction (RT-PCR) to examine gene expression of cytokines, including IL-1ß, IL-6, IL-8, TGF-ß, IL-4, IL-5, and IFN-γ.

MATERIALS AND METHODS

Subjects. Nasal polyp tissues were taken from 14 subjects (10 men, 4 women, 24 to 60 years of age) undergoing polypectomy for nasal obstruction after obtaining informed consent. Among them, 4 patients (3 men, 1 woman, 33 to 54 years of age) suffered from associated perennial allergic rhinitis, as diagnosed by clinical history, positive skin prick test, and/or radioallergosorbent test (RAST) to house dust mites (*Dermatophagoides pteronyssinus* or *Dermatophagoides farinae*), the most common offending allergen in Korea. The remaining 10 patients had no history of nasal allergy, asthma, or aspirin sensitivity and showed negative reactions to the skin test and/or RAST to house dust mites. Patients were excluded if they had previously received surgery, or had taken regular topical or oral medication within 3 weeks. The patients were not studied within 3 weeks after recovering from an upper respiratory tract infection. The turbinate mucosae from 6 normal subjects were used as controls after obtaining informed consent. This study was approved by the Institutional Review Board of Seoul National University Hospital. To assess the cytokine gene expression, the tissues were frozen immediately after removal in liquid nitrogen and were stored at –70°C for subsequent RNA isolation.

RT-PCR. Frozen tissues were homogenized and total RNA was extracted by the guanidinium-phenol-chloroform extraction method.[15] Finally, the RNA pellet was resuspended in 50 mL of diethylpyrocarbonate (DEPC). As a positive control, we used complementary DNA (cDNA; Clontech Laboratories, Palo Alto, Calif) of each cytokine. The concentration of total RNA was determined by measuring the optical density at 260 nm, and the integrity of extracted RNA was confirmed by comparing the 28S and 18S bands on denaturing gel electrophoresis.

Each RNA solution prepared as above was reverse-transcribed into cDNA with a Gene-Amp RNA-PCR kit (Perkin-Elmer Cetus Corp, Branchburg, NJ). Five millimolar magnesium chloride ($MgCl_2$), 1 mmol/L dNTP (dATP, dCTP, dGTP, dTTP), 2 mL of 10X PCR buffer II, 1 mL of DEPC-treated distilled water, 1 unit of RNase inhibitor, 2.5 mmol/L random hexamer, and 2.5 units of murine leukemia virus reverse transcriptase were added to 1 mg of total RNA. This RNA mixture was incubated at room temperature for 10 minutes, at 42°C for 15 minutes, and at 95°C for 5 minutes and then cooled to 5°C. The PCR was carried out in a DNA thermal cycler (Perkin-Elmer Cetus Corp). Twenty milliliters of cDNA were amplified with Ampli-Taq DNA polymerase (Perkin-Elmer Cetus Corp) according to the manufacturer's PCR protocol. The PCR mixture consisted of 2 mmol/L $MgCl_2$, 8 mL of 10X PCR reaction buffer II, 0.5 mL of AmpliTaq DNA polymerase (2.5 U/100 mL), 0.2 mmol/L downstream and upstream primers (Clontech Laboratories), and 65.5 mL of sterile water. This mixture was mixed with 20 mL of reverse-transcribed products to a final volume of 100 mL. After overlaying the reaction mixture with 40 mL of light mineral oil (Sigma M-3516), hot-start PCR was carried out for 35 cycles. The conditions for PCR were as follows: 1 minute at 95°C for the denaturation step, 1 minute at 55°C for the annealing step, and 1 minute at 72°C for the extension step. Primer sequences of each cytokine and ß-actin used in this study are listed in Table 1. The predicted sizes of amplified fragments of IL-1α, IL-1ß, IL-6, IL-8, TGF-ß, IL-4, IL-5, IFN-γ, and ß-actin were 491, 802, 628, 298, 161, 456, 294, 427, and 838 base pairs, respectively. Control cDNAs of each cytokine were used as positive controls, and ß-actin (Clontech Laboratories) was used as an internal control in the PCR procedure. As a negative control, sterile distilled water was added to the reaction mixture instead of cDNA at the reverse transcription step. Specificity of the PCR product for each cytokine was detected by Southern blot analysis.

Southern Blot Analysis of Amplified cDNA. Digoxigenin-labeled probe was synthesized with DNA polymerase. Control cDNA of each cytokine was used as a template, and digoxigenin-dUTP was added to the PCR reaction mixture instead of dUTP. Hybridization solution was prepared by mixing 50% formamide with 5X standard saline citrate (SSC), 2% blocking solution (Boehringer Mannheim, Mannheim, Germany), 0.1% N-lauroyl sarcosine, and 0.02% sodium dodecyl sulfate (SDS). Amplified cDNA was separated by electrophoresis through a 1.5% agarose gel and was transferred to nylon membrane by the capillary diffusion method. Cross-linking of DNA was performed by drying at 80°C in a vacuum for 2 hours. A nylon membrane containing target DNA was sealed with hybridization solution and incubated for 3 hours at 42°C. After adding the denatured probe to the hybridization solution, the sealed bag was incubated in a shaking water bath for 16 hours at 42°C. The condition for washing was as follows: 2X SSC and 0.1% SDS at room temper-

TABLE 1. PRIMER SEQUENCES

	Upstream (5′ Primer)	*Downstream (3′ Primer)*
IL-1α (491 bp)	5'CAAGGAGAGCATGGTGGTAGTAGCAACCAACG3'	5'TAGTGCCGTGAGTTTCCCAGAAGAAGAGGAGG3'
IL-1β (802 bp)	5'ATGGCAGAAGTACCTAAGCTCGC3'	5'ACACAAATTGCATGGTGAAGTCAGTT3'
IL-6 (628 bp)	5'ATGAACTCCTTCTCCACAAGCGC3'	5'GAAGAGCCCTCAGGCTGGACTG3'
IL-8 (298 bp)	5'ATGACTTCCAAGATGGCCGTGGCT3'	5'TCTCAGCCCTCTTCAAAAACTTCTC3'
TGF-β (161 bp)	5'GCCCTGGACACCAACTATTGC3'	5'AGGCTCCAAATGTAGGGGCAGG3'
IL-4 (456 bp)	5'ATGGGTCTCACCTCCCAACTGCT3'	5'CGAACACTTTGAATATTTCTCTCTCAT3'
IL-5 (294 bp)	5'GCTTCTGCATTTGAGAGTTTGCTAGCT3'	5'GGCCGTCAATGTATTTCTTTATTAAG3'
IFN-γ (427 bp)	5'GCATCGTTTTGGGTTCTCTTGGCTCTTACTGC3'	5'CTCCTTTTTCGCTTCCCTGTTTTAGCTGCTGG3'
β-actin (838 bp)	5'ATCTGGCACCACACCTTCTACAATGAGCTGCG3'	5'CGTCATACTCCTGCTTGCTGATCCACATCTGC3'

ature for 5 minutes, repeated two times, and then, 0.1X SSC and 0.1% SDS at 68°C for 15 minutes two times. Dig DNA labeling and detection kit (Boehringer Mannheim) for nucleic acids was used to detect the target DNA by colorimetry. The nylon membrane containing target DNA bound with the probe was kept in blocking reagent at room temperature for 30 minutes and then incubated in a mixture of blocking solution and alkaline phosphatase–conjugated antidigoxigenin antibody at room temperature for 30 minutes. Nitroblue tetrazolium chloride/X–phosphate, 4-toluidine salt solution was applied for 30 minutes in a dark place to develop the color reaction.

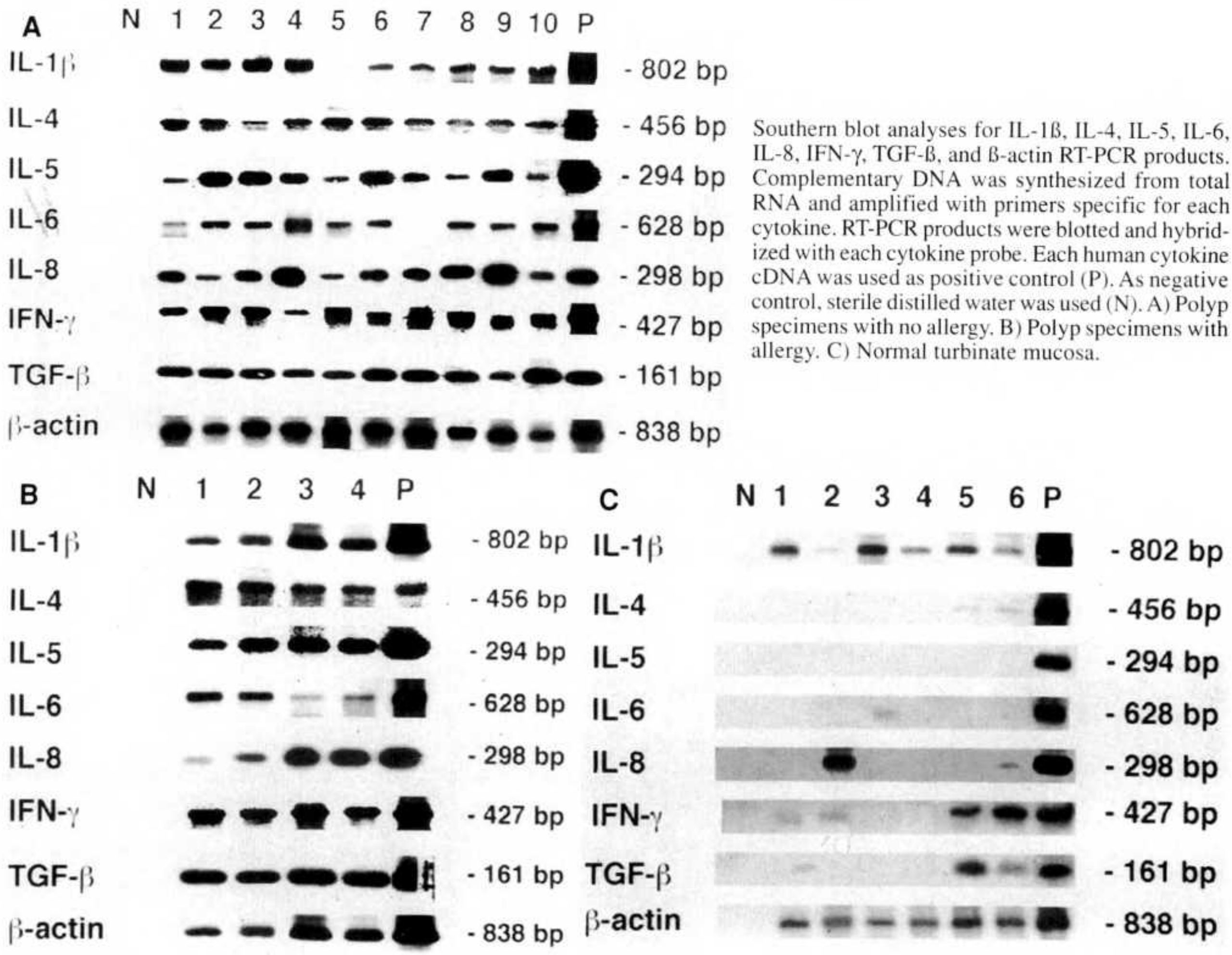
Quantitation of Positive Band Intensity. Band intensity of amplification products for each cytokine and ß-actin obtained from RNA of individual subjects was quantitated with a densitometer. To compare the bands' intensity between polyps with allergy and polyps without allergy, the density ratio (cytokine/ß-actin) was calculated by dividing the intensity of the band of each cytokine by that of ß-actin in the same specimen.[16]

RESULTS

To investigate the pattern of cytokine gene expression in nasal polyp tissues, we conducted RT-PCR followed by Southern blot hybridization. We were able to detect expression of various cytokine genes in nasal polyp tissues. The specific bands of IL-1ß, IL-8, and TGF-ß mRNAs were observed in all the polyp specimens (see Figure, A,B). Nine of 10 polyp specimens with no allergy expressed a signal for IL-6 mRNA (see Figure, A), and all of 4 polyp tissues with allergy showed signal for IL-6 (see Figure, B), whereas in normal subjects, only IL-1ß mRNA was universally expressed, and IL-6, IL-8, and TGF-ß mRNAs could be detected in 1, 2, and 3 of 6 normal turbinate mucosae, respectively (see Figure, C).

We detected signals on Southern blot specific for IL-4 and IL-5, Th2-type cytokines known to be important in the pathogenesis of allergy. Interferon-γ mRNA, an inhibitor of IL-4 function, was also expressed in nasal polyps. However, these cytokine mRNAs were expressed in all the specimens, irrespective of the presence or absence of allergy (see Figure, A,B). In contrast, the mRNAs of IL-4, IL-5, and IFN-γ were observed in 2, 0, and 4 of 6 normal turbinate mucosae, respectively (see Figure, C). Interleukin-6, IL-8, TGF-ß, IL-4, IL-5, and TGF-ß mRNAs were expressed more frequently in polyp specimens than in normal turbinate mucosa. However, there were no differences in the expression of IL-1ß mRNA between normal subjects and patients with nasal polyps. When sterile water was used as a negative control, no amplified DNA was detected. The RT-PCR products of RNA from all 14 nasal polyp specimens examined expressed a positive band to ß-actin probe.

As an alternative way to compare the results quantitatively, the cytokine/ß-actin ratios were calculated in different groups, and they are shown in Table 2. There were no differences in mean density ratios for IL-1ß and TGF-ß between normal turbinate mucosa and nasal polyps, regardless of allergy. All the polyp specimens revealed a relatively higher mean density ratio for each cytokine investigated than normal



Southern blot analyses for IL-1ß, IL-4, IL-5, IL-6, IL-8, IFN-γ, TGF-ß, and ß-actin RT-PCR products. Complementary DNA was synthesized from total RNA and amplified with primers specific for each cytokine. RT-PCR products were blotted and hybridized with each cytokine probe. Each human cytokine cDNA was used as positive control (P). As negative control, sterile distilled water was used (N). A) Polyp specimens with no allergy. B) Polyp specimens with allergy. C) Normal turbinate mucosa.

turbinate mucosa, except IL-1ß and TGF-ß. However, there were no differences in mean density ratios of each cytokine between polyps with allergy and polyps without allergy. The mean density ratios for IFN-γ, IL-6, and IL-8 in all the nasal polyps were relatively higher than those for other cytokines.

DISCUSSION

Information on the expression of cytokine genes in the nasal polyp tissues is still limited. Ohno et al reported that granulocyte-macrophage colony stimulating factor[17] and TGF-ß[18] were expressed in nasal polyp tissues, but not in normal mucosa. Liu et al[19] quantified IL-1 and suggested that the major source of IL-1 seemed to be mononuclear leukocytes in nasal polyp tissues. However, each of these reports described expression of one or two cytokines in nasal polyps, and very little information is available on the pattern of cytokine expression in nasal polyps. It is important to clarify the patterns of cytokine gene expression in nasal polyp tissue, because it is generally known that several cytokines are involved in the process of inflammatory reaction in vivo.

All the cytokines examined in this study — IL-1ß,

TABLE 2. MEAN DENSITY RATIO OF POSITIVE BAND FOR EACH CYTOKINE

Cytokines	*Turbinate Mucosa (N = 6)*	*Polyps With Allergy (N = 4)*	*Polyps Without Allergy (N = 10)*
IL-1β	0.93 ± 0.34 (6)	0.92 ± 0.22 (4)	0.93 ± 0.22 (10)
IL-6	0.73 ± 0.00 (1)	1.60 ± 0.61 (4)	1.69 ± 0.87 (9)
IL-8	0.86 ± 0.07 (2)	1.58 ± 0.49 (4)	1.39 ± 1.29 (10)
TGF-β	0.72 ± 0.21 (3)	0.78 ± 0.08 (4)	0.85 ± 0.12 (10)
IL-4	0.39 ± 0.09 (2)	0.87 ± 0.12 (4)	0.73 ± 0.26 (10)
IL-5	0 (0)	0.77 ± 0.18 (4)	0.65 ± 0.06 (10)
IFN-γ	0.62 ± 0.15 (4)	2.28 ± 0.72 (4)	2.21 ± 1.39 (10)

In parentheses are numbers of positive bands on Southern blot.

IL-6, IL-8, TGF-ß, IL-4, IL-5, and IFN-γ — expressed their mRNA genes in virtually all of the nasal polyp specimens, whereas only IL-1ß mRNA was expressed in all 6 normal mucosae. These results suggest that the various cytokines are produced in nasal polyp tissues and that they may play important roles in the formation of nasal polyps. Of the cytokines studied, the mean density ratios of three cytokines — IL-6, IL-8, and IFN-γ — were higher than those of other cytokines. However, further investigation is needed to evaluate its significance.

Many reports have proposed an allergic mechanism in the pathogenesis of nasal polyps. Nasal polyp tissues have a large amount of histamine and have eosinophil infiltration[20,21]; these findings suggest that an allergic mechanism may contribute to the development of nasal polyps. Hamilos et al[22] studied the cytokine expression in allergic versus nonallergic chronic sinusitis with nasal polyps using in situ hybridization and proposed that different mechanisms of eosinophilia exist in patients with allergic and nonallergic chronic sinusitis with nasal polyps. They suggested that nonallergic eosinophilia in nasal polyps is independent of both IL-4 and IL-5, cytokines that contribute to tissue eosinophilia in allergic inflammation.

In contrast, IL-4 and IL-5 mRNAs were equally expressed in the nasal polyps from nonallergic subjects, as well as those from allergic subjects, in our study. These results suggest that allergic mechanisms may not play important roles in the pathogenesis of nasal polyps, since IL-4 and IL-5 are key cytokines in the pathogenesis of allergy. Further, mRNA of IFN-γ antagonistic to IL-4 was also expressed in all of 14 nasal polyps. From these results, we suggest that eosinophilia in nasal polyps may be dependent on IL-4 and IL-5, and that IL-4 and IL-5 mRNAs in nasal polyp could be induced through yet-to-be-identified immunologic mechanisms other than type I allergy. Our study is compatible with other studies that propose that allergy may not be the cause of nasal polyps.[4,23]

Some cytokines have been reported to be produced by eosinophils.[17,18,24] In nasal polyps and asthma, eosinophils are often found in and around the vessels and the epithelium. The cytokines investigated in this study have been implicated as mediators of inflammatory reactions. Interleukin-4 stimulates the expression of vascular cell adhesion molecule–1 on endothelial cells, resulting in increased binding of lymphocytes, monocytes, and especially eosinophils. Interleukin-5 increases the survival of eosinophils. These two cytokines are probably responsible for high levels of infiltration of eosinophils in nasal polyps, although other cytokines, such as RANTES (Regulated on Activation, Normal T cells Expressed and Secreted), may also play a role in the recruitment of eosinophils in nasal polyps. The principal function of IL-1, IL-6, and IL-8 is to mediate the host inflammatory response in natural immunity. Interleukin-1 upregulates the expression of cell adhesion molecules in endothelial cells, thus promoting leukocyte adhesion to blood vessels.[25,26] In this study, IL-1ß was expressed even in normal mucosa, and there was no difference in IL-1ß gene expression between nasal polyp and healthy turbinate mucosa. This means that IL-1ß may be constitutively expressed in nasal mucosa. Our data pose an interesting question about the role of IL-1ß in normal nasal mucosa in addition to its role in the inflammatory process. Interleukin-6 is another multifunctional cytokine secreted by a number of cells, including T cells, macrophages, eosinophils, and activated T cells.[27] Interleukin-8 is predominantly a chemoattractant for neutrophils,[28] as well as for eosinophils after the cells have been primed with IL-5.[29] Thus, in addition to IL-4 and IL-5, IL-8 may contribute to eosinophil infiltration in nasal polyps. Because TGF-ß is a chemoattractant for fibroblasts[30] and stimulates fibroblast proliferation,[31] it may play a role in the pathogenesis of nasal polyps. There appears to be no single etiologic factor in the pathogenesis of nasal polyps, and the formation of nasal polyps may be the common final result of a variety of different and unrelated mechanisms.

In conclusion, all of the cytokine genes studied — IL-6, IL-8, TGF-ß, IL-4, IL-5, and IFN-γ mRNAs — were detected more frequently in polyp specimens than in normal turbinate mucosa, with the exception of IL-1ß mRNA, which showed no difference between nasal polyps and normal turbinate mucosa. Our data suggest that many individual cytokine genes are expressed in nasal polyp tissue and may play important roles in the pathogenesis of nasal polyps. All of the cytokine mRNAs studied were expressed in nasal polyps, irrespective of the presence or absence of allergy. This result suggests that allergy per se may not be the cause of polyp formation, and that the results of an inflammatory reaction in the nasal mucosa induced by a complex array of cytokines may lead to secondary formation of nasal polyps.

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ASPIRIN, NONSTEROIDAL ANTI-INFLAMMATORY DRUGS, AND EPISTAXIS

A REGIONAL RECORD LINKAGE CASE CONTROL STUDY

HUEY L. TAY, FRCS
JOSIE M. M. EVANS, MA (OXON)
ALEXANDER D. MCMAHON, BSC
THOMAS M. MACDONALD, MD, FRCP
DUNDEE, SCOTLAND

To assess the relationship between nonsteroidal anti-inflammatory drugs (NSAIDs) and spontaneous epistaxis in adults over 50 years old, a case control study was carried out by using a record linkage database for the population of Tayside, Scotland, which included 319,465 people. The study group consisted of 326 patients who were hospitalized with epistaxis between May 1989 and December 1992, but who had not previously been hospitalized with this diagnosis. Six community controls and 4 hospital controls, matched for age and sex to each case, were used. Previous exposure to prescribed aspirin and other NSAIDs was investigated. There was a significant association between aspirin exposure and epistaxis when either community or hospital controls were used ($p < .001$). Patients who had aspirin prescriptions had a relative risk of hospital admission for epistaxis of between 2.17 and 2.75, depending on the control group used. No association between non-aspirin NSAIDs and epistaxis was evident with either control group.

KEY WORDS — aspirin, case control study, epistaxis, nonsteroidal anti-inflammatory drugs.

INTRODUCTION

Epistaxis is a common otolaryngological emergency that often necessitates hospital admission, particularly among older patients. In the majority of these patients, the bleeding arises from an artery or vein without any obvious cause — hence the term "spontaneous epistaxis." Known etiologic factors include nasal trauma, blood dyscrasia, blood vessel abnormality, and nasal tumor.

Aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) induce platelet dysfunction, primarily by inhibition of thromboxane synthesis.[1] In a case control study comparing 53 epistaxis patients with 50 controls, Watson and Shenoi[2] found an association between the use of NSAIDs and the occurrence of epistaxis. This association was confirmed by Livesey et al[3] in a study of the same format.

Although these studies suggested an association between NSAID use and spontaneous bleeding, they were by no means definitive. It is possible that the underlying illness for which NSAIDs are taken may predispose to bleeding. It is well known that the antiplatelet effect of aspirin is utilized clinically in prophylaxis to decrease the incidence of myocardial infarction, stroke, venous thromboembolism, and peripheral vascular graft failures.[4-6] In contrast, the indications for non-aspirin NSAIDs (NA-NSAIDs) tend to be osteoarthritis, chronic inflammatory disease, and musculoskeletal complaints.

To clarify the risk of epistaxis associated with aspirin and NA-NSAIDs, we carried out a case control study with multiple control groups, using a record linkage database for a population of 319,465 people in Tayside, Scotland.

PATIENTS AND METHODS

The study was carried out using the record linkage database of the Tayside Medicines Monitoring Unit (MEMO) at the University of Dundee. This database was developed for pharmacoepidemiological research.[7,8] It contains prospectively gathered information on drugs that have been dispensed in community pharmacies for the Tayside population from January 1989. This information is patient-specific and indexed by the Community Health Number (CHNo), a unique 10-digit number that is routinely allocated to all patients when they register with a general practitioner (GP) in Scotland. The prescription database can be linked to a database of inpatient hospital activity, which is also indexed by the CHNo. For every consultant episode of care in Tayside from 1980, administrative data (eg, date of admission, date of discharge), demographic data (eg, age and sex of patient), and diagnostic data (up to six ICD9 codes[9]) are available. Studies into drug safety have been performed by linking the prescription and hospital activity databases via the CHNo.[10-13] In this particular study, records of prescriptions dispensed for aspirin and NA-NSAIDs were linked to records of hospital

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From the Departments of Otolaryngology (Tay) and Clinical Pharmacology (Evans, McMahon, MacDonald), Ninewells Hospital, Dundee, Scotland.

CORRESPONDENCE — Josie M. M. Evans, MA (Oxon), Medicines Monitoring Unit, Ninewells Hospital, Dundee DD1 9SY, Scotland.

TABLE 1. COMMUNITY CONTROLS AND DRUG EXPOSURE

	Cases *(n = 326)*		*Controls* *(n = 1,956)*				
Drug	*No.*	*%*	*No.*	*%*	*Odds Ratio*	*95% CI*	*p*
Aspirin							
Within 50 days	30	9.2	77	3.9	2.47	1.59, 3.84	<.001
Within 100 days	38	11.6	91	4.7	2.75	1.83, 4.12	<.001
NA-NSAID							
Within 50 days	40	12.3	192	9.8	1.30	0.89, 1.87	.169
Within 100 days	54	16.6	265	13.5	1.28	0.92, 1.78	.136
Cardiovascular							
Ever	144	44.2	631	32.3	1.70	1.33, 2.18	<.001
Calcium channel blockers							
Ever	33	10.1	109	5.6	1.89	1.26, 2.84	.002

CI — confidence interval, NA-NSAID — non-aspirin nonsteroidal anti-inflammatory drug.

admissions for epistaxis.

The study population, from which the patients and controls were chosen, comprised 319,465 people who were resident in Tayside and registered with a Tayside GP in January 1989, and were either still resident in October 1994 or had died (in Tayside) during this period.

Patients and Controls. A patient was defined as a case within the database if he or she had an emergency hospital admission with a primary diagnosis of epistaxis between May 1, 1989, and December 31, 1992, inclusively, and had no previous hospital admission for this diagnosis prior to May 1, 1989. The cases were therefore incident cases of epistaxis. Only those patients 50 years old or over at the time of admission were selected. The ICD9 code used to identify the cases was 784.7 (epistaxis). None of the patients had been admitted to hospital at any time since 1980 with any of the following ICD9 diagnoses: coagulation defects (286.0, 286.1, 286.2, 286.3, 286.9), von Willebrand's disease (286.4), or primary thrombocytopenia (287.3). The assumption was therefore that the patients had spontaneous epistaxis, and had no underlying predisposition to epistaxis.

From the study population, 6 community controls, matched for age and sex, were generated at random for each case of epistaxis. The date of the case's first hospital admission was taken as the index date for its matched controls. Four hospital controls, matched for age, sex, and hospital admission date, were also generated for each case. These controls had hospital admissions with a diagnosis other than epistaxis within 90 days of their corresponding epistaxis case. The hospital departments to which these controls had been admitted included otolaryngology, ophthalmology, oral surgery, neurosurgery, plastic surgery, orthopedics, gynecology, urology, rehabilitation–geriatric medicine, dermatology, and respiratory medicine.

Analysis. For both cases and controls, previous exposure to oral aspirin and oral NA-NSAIDs was investigated. Two predefined exposure variables were used — 50 days and 100 days — defined as whether one or more prescriptions of the specific drug was dispensed (exposure) during a 50- or 100-day period prior to the index date. Odds ratios and χ^2 were calculated for each of the exposure variables with conditional logistic regression. Statistical analyses were performed with SAS version 6.10 for Microsoft Windows.[14]

Aspirin prescriptions in the community might be associated with other cardiovascular drug use, because patients who take aspirin are likely to do so for prophylaxis of heart attack, stroke, or peripheral vascular diseases. Patients exposed to cardiovascular drugs may already be predisposed to epistaxis, so exposure to these drugs might confound the association between aspirin and epistaxis. Therefore, "ever exposure" to drugs acting on the cardiovascular system was investigated in a nested case control analysis among patients who had all been exposed to aspirin. The cardiovascular drugs included were those defined in the *British National Formulary*[15] (chapter 2). Any exposure from January 1, 1989, to the index date was used for both aspirin and cardiovascular drugs ("ever exposure") in this part of the analysis. Twelve new community controls who had all been exposed to aspirin were generated for each aspirin-exposed case. This analysis was then repeated for "ever exposure" specifically to calcium channel blockers (chapter 2.6.2 of the *British National Formulary*).

RESULTS

The cases consisted of 151 men and 175 women with a mean age of 71 years (range 50 to 90 years). Suitable community controls were found for all of

TABLE 2. HOSPITAL CONTROLS AND DRUG EXPOSURE

	Cases (n = 302)		*Controls (n = 1,203)*				
Drug	*No.*	*%*	*No.*	*%*	*Odds Ratio*	*95% CI*	*p*
Aspirin							
Within 50 days	30	9.9	60	5.0	2.17	1.35, 3.47	.001
Within 100 days	37	12.3	75	6.2	2.19	1.42, 3.37	<.001
NA-NSAID							
Within 50 days	37	12.3	141	11.7	1.05	0.71, 1.56	.791
Within 100 days	50	16.6	195	16.2	1.03	0.73, 1.45	.873
Cardiovascular							
Ever	133	44.0	463	38.5	1.27	0.98, 1.66	.071
Calcium channel blockers							
Ever	32	10.6	94	7.8	1.42	0.92, 2.18	.113

the cases. However, no suitable hospital controls could be found for 24 cases, which were excluded from the statistical analyses.

There was a significant association between aspirin exposure and hospital admission for epistaxis, regardless of the control group and the exposure variable used (Tables 1 and 2). Patients who had aspirin prescriptions had an estimated relative risk of hospital admission for epistaxis of between 2.17 (95% CI = 1.35, 3.47) and 2.75 (95% CI = 1.83, 4.12). However, there was no association between the use of NA-NSAIDs and epistaxis.

Previous exposure to cardiovascular drugs appeared to be associated with epistaxis as compared to community controls ($p < .001$), and there was a trend with hospital controls ($p = .071$). There was also an association between the use of calcium channel blockers and epistaxis with community controls ($p = .002$). Table 3 provides the results of the nested case control study of exposure to cardiovascular drugs and calcium channel blockers in patients and controls who all had aspirin exposure. It shows that there is no independent association between epistaxis and exposure to cardiovascular drugs ($p = .369$) or calcium channel blockers ($p = .921$) in this population.

DISCUSSION

Platelet dysfunction induced by NSAIDs is related primarily to inhibition of thromboxane synthesis.[1] Platelet activation by a variety of factors results in the release of platelet granules and the recruitment of other platelets into the primary hemostatic plug.[16] The degree of platelet dysfunction of NSAIDs is variable and may be related to both the quantitative and qualitative inhibition of thromboxane synthesis. Aspirin differs from all other NSAIDs in that it produces irreversible acetylation of cyclooxygenase. Hence, its antiplatelet effect is permanent and lasts for 5 to 7 days of the life of the platelet.[1] In contrast, NA-NSAIDs have a reversible but variable degree of inhibition of this enzyme.[17] These differences between aspirin and NA-NSAIDs are therefore important in the study of the risk of spontaneous bleeding such as epistaxis.

Our results show that while there is an association between the use of aspirin and hospital admissions for epistaxis, there is no significant association between exposure to NA-NSAIDs and epistaxis. This difference has several possible explanations. The potency and qualitative difference in platelet dysfunction caused by aspirin compared to NA-NSAIDs seems to be the most plausible explanation. However, it is possible that the underlying illness for which aspirin is taken may also predispose to bleeding. Thus, epistaxis might be related to the increased incidence of arteriosclerosis or hypertension in patients on long-term aspirin regimens.

We addressed this possible confounding effect by carrying out a nested case control analysis of cardiovascular drugs in the population of patients taking aspirin. If the association between aspirin and epistaxis was due to aspirin-exposed patients also having disorders for which they were treated with cardiovascular drugs, an association between "ever exposure" to cardiovascular drugs and epistaxis would

TABLE 3. "EVER EXPOSURE" TO CARDIOVASCULAR DRUGS OR CALCIUM CHANNEL BLOCKERS AND EPISTAXIS

	Cases (n = 70)		*Controls (n = 840)*				
	No.	*%*	*No.*	*%*	*Odds Ratio*	*95% CI*	*p*
Cardiovascular drugs	52	74.3	582	69.3	1.29	0.73, 2.29	.369
Calcium channel blockers	13	18.6	160	19.0	0.97	0.51, 1.84	.921

also be evident in the population of patients exposed to aspirin. This was not the case. So, aspirin exposure seems to be independently associated with hospitalization for epistaxis. The same argument can be applied for "ever exposure" to calcium channel blockers. It is important to note that the risks for aspirin in this study apply to prescribed aspirin only, the majority of which is low-dose in Tayside. No information was available on exposure to aspirin purchased over the counter.

There is at present no convincing evidence to suggest that NA-NSAIDs, as a whole group, carry an increased risk of severe epistaxis. However, there might be a subgroup of NA-NSAIDs that have high platelet dysfunction potency, and further studies in this area are necessary.

There is no doubt that it is cost-effective to treat high-risk vascular patients with daily aspirin.[4] This benefit far outweighs the risk of epistaxis. However, this cost-effectiveness would need to be questioned if aspirin is recommended for treating low-risk individuals. Studies have shown that aspirin, even on low dosage, increases the risk of gastrointestinal and intracranial hemorrhage.[4,18] The self-medicating general public on aspirin should also be made aware of the significant risk of bleeding.

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EFFECT OF SUBSTANCE P ON THE SHORT-CIRCUIT CURRENT OF RAT NASAL MUCOSAL EPITHELIUM

PING-CHANG YANG, MD, PHD
DUNDAS, CANADA
TAO-YUAN ZHANG, MD
TAIYUAN, PEOPLE'S REPUBLIC OF CHINA
TAO LIU, MD
TAIYUAN, PEOPLE'S REPUBLIC OF CHINA
DAO-SHENG FAN, MD
TAIYUAN, PEOPLE'S REPUBLIC OF CHINA

Rats were sensitized by intranasal application of toluene diisocyanate as a nasal allergy model. By means of the Ussing chamber technique, rat nasal epithelial short-circuit current (Isc) was measured. Enhanced Isc of the rat nasal mucosa resulted from stimulation of substance P (SP) in a dose-dependent manner that could be inhibited by pretreatment with NK1 receptor antagonist CP-96345, the H1 receptor antagonist pyrilamine, the H2 receptor antagonist ranitidine, and the neurotoxin tetrodotoxin, respectively, to different extents. The results indicate that SP is able to cause ion secretion of the nasal mucosal epithelium, perhaps by activating mast cells to release histamine. These data suggest that mast cells and sensory nerves participate in the regulation of SP-induced ion secretion during nasal allergy.

KEY WORDS — mast cell, nasal allergy, nasal mucosa, sensory nerve, substance P.

INTRODUCTION

Nasal allergy often results in abnormal nasal function, including excess nasal gland secretion, nasal mucosal swelling, increase in blood vessel permeability, and higher nasal epithelial permeability.[1] The mechanisms of change of these nasal functions have not been fully understood. It is suggested that the antidromic release of neuropeptides such as substance P (SP) from the peripheral nerve endings of sensory nerves in the airways contributes to airway hyperresponsiveness.[2] These neuropeptides such as SP, which is found in the dense network of sensory nerves in the nasal mucosa, may thus play an important role in the pathophysiology of nasal allergy.[3] The morphologic association between mast cells and unmyelinated nerve fibers has been established with electron microscopy, and there is also accumulating evidence for a functional relationship between mast cells and sensory nerves, in which SP causes histamine release from rat mast cells.[4]

Therefore, we carried out the present study to examine the role of the neuropeptide SP in nasal allergy. We used rats sensitized by repeated administration of toluene diisocyanate (TDI) to induce nasal allergy–like behaviors: sneezing and watery rhinorrhea.[5] We then examined nasal epithelial short-circuit current (Isc) stimulated by SP, which was potentiated by alteration of ion secretion of the mucosal epithelium, by using the classic Ussing chamber technique.[6]

MATERIAL AND METHODS

Male Sprague-Dawley rats (250 to 300 g) were housed in separate quarters in standard rat cages equipped with filter hoods in an environment controlled for temperature (20°C) and photoperiod (12:12 hour light-dark cycle) with free access to food and water. The animals were handled daily before experiments were performed. The rats were sensitized to 10 μL of a solution of 10% TDI (Wako, Japan) in ethyl acetate, which was painted bilaterally on the nasal vestibuli of the animals once a day for 5 consecutive days. This sensitization process was carried out again 3 weeks after the first immunization. One week after the second immunization, 10 μL of 5% TDI solution was applied to the nasal vestibuli to induce nasal allergy–like symptoms in the TDI-sensitized animals. Provocation was executed with 5% TDI. Control animals were treated with normal saline by the same procedure.

Experiments were conducted 2 days after the last provocation with 5% TDI. Rats were deeply anesthetized by 1 mL/100 g body weight 50% urethane intramuscularly. A vertical incision was made in the midline and carried down to the periosteum of the nasal bone to expose the nasal bones bilaterally. With a diamond drill, under a surgical microscope, the nasal bones were cut off, with caution not to damage any nasal mucosa; we elevated and separated the nasal mucosa from the nasal septum, and extended it to a part of the nasal floor to make sure the nasal mucosal strip was larger than 1 × 1.2 cm in size. The mucosal strip was placed into prewarmed (37°C) Krebs buffer immediately after it was cut off from the nasal cavity, then mounted in an Ussing chamber, exposing 0.6 cm^2 of epithelial surface area to Krebs

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From the Department of Otolaryngology, First Affiliated Hospital, Shanxi Medical University, Taiyuan, People's Republic of China.
CORRESPONDENCE — Ping-Chang Yang, MD, PhD, 15 Don St, Apt 14, Dundas, Ontario, Canada L9H 4P1.

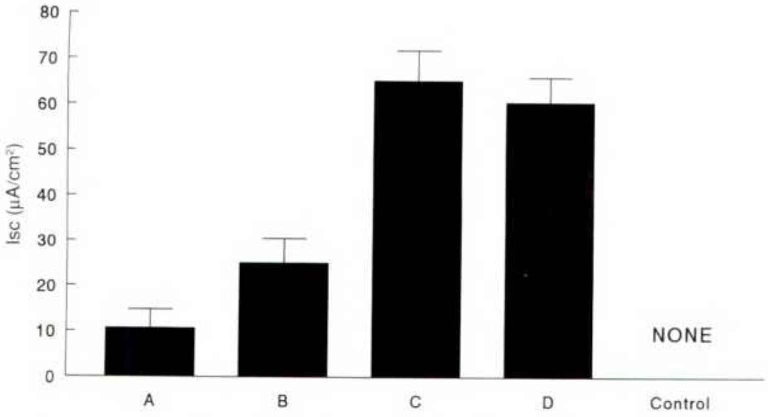


Fig 1. Short-circuit current (Isc) changes of sensitized rat nasal mucosal epithelial tissue caused by stimulation of substance P (SP). Increase in Isc could be elicited by SP at concentration of 10^{-8} mol/L; it kept increasing while SP concentration went up. Peak value of Isc was reached when stimulated by 10^{-6} mol/L SP. Concentrations of SP were group A, 10^{-8} mol/L; group B, 10^{-7} mol/L; group C, 10^{-6} mol/L; and group D, 10^{-5} mol/L. Control group, naive rats, showed no Isc response to 10^{-6} mol/L SP stimulation.

buffer (in mmol/L: 115 sodium chloride, 8 potassium chloride, 1.25 calcium chloride, 1.2 magnesium chloride, 2 potassium hydrogen phosphate, 25 sodium hydrogen phosphate, pH 7.35 ± 0.02) containing 10 mmol/L mannitol on the epithelial side chamber and osmotically balanced with 10 mmol/L glucose on the submucosal side chamber. A circulating water bath maintained the buffers at 37°C. The current crossing the tissue was measured in the voltage-clamp mode (zero potential difference) and expressed as short-circuit current (Isc, $\mu A/cm^2$).

The SP stimulation group consisted of 20 rats sensitized to TDI and randomized into 4 subgroups designated groups A, B, C, and D. Another 5 naive rats were used as a control. The SP stimulation was conducted by adding SP into the epithelial surface side chamber to make the final concentration — group A, 10^{-8}; group B, 10^{-7}; group C, 10^{-6}; and group D, 10^{-5} mol/L — in order to examine the effects of various concentrations of SP. The control group specimens were stimulated by addition of a single concentration of 10^{-6} mol/L SP. The Isc response to the stimulation of SP was registered by a computer automatically.

Blocking Effects of Various Agents on Isc Caused by SP. Thirty-five rats sensitized by TDI were randomized into 7 groups designated as groups E, F, G, H, I, J, and K. Groups E, F, and G were used to examine whether SP-induced ion secretion was mediated by an NK_1 receptor. Tissues were pretreated with the selective nonpeptide NK_1 antagonist CP-96345 ([(2s,3s)-cis-2-(diphenylmethyl)]-N-[(2-methoxyphenyl)-methyl]-1-azabicyclo[2,2,2]octan-3-amine; Sigma) in the following concentrations — group E, 5×10^{-6} mol/L; group F, 10^{-5} mol/L; and group G, 5×10^{-4} mol/L — for 15 minutes before adding the stimulator of 10^{-6} mol/L SP. Groups H and I were used to investigate whether the increase in Isc response to 10^{-6} mol/L SP was mediated by histamine release from mast cells; two histamine receptor antagonists were used in this study. Group H nasal epithelial specimens were pretreated by the H_1 receptor antagonist pyrilamine (5×10^{-6} mol/L, Sigma), and group I specimens, by the H_2 receptor antagonist ranitidine (5×10^{-6} mol/L, Sigma) for 15 minutes before stimulation with 10^{-6} mol/L SP. To examine the effects of nasal sensory nerves in the response to SP, we used the neurotoxin tetrodotoxin (TTX, Sigma) at 10^{-6} mol/L to pretreat the specimens of group J for 15 minutes before stimulation by adding 10^{-6} mol/L SP. Specimens of group K were pretreated with the H_1 receptor antagonist pyrilamine at the concentration of 5×10^{-7} mol/L, combined with the neurotoxin tetrodotoxin at 10^{-6} mol/L. After pretreatment with these antagonists and inhibitors for 15 minutes in an Ussing chamber, the specimens were stimulated by adding SP to the epithelial side chamber at the final concentration of 10^{-6} mol/L.

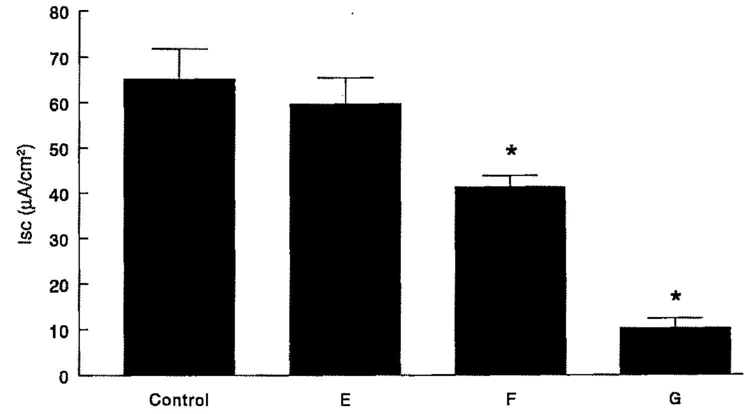
Data Analysis. The results were expressed as mean ± SD. Results from the two tissue strips of both sides of the nasal septum were pooled and we took the average from each animal. Student's *t*-test was used to compare the difference in results of the test group and the control group, with the level of statistical significance set at $p < .05$.

RESULTS

After equilibration for 15 minutes in an Ussing chamber, the specimens were stimulated by SP. At the concentration of 10^{-8} mol/L, SP evoked the Isc responses. The Isc responses kept increasing in a dose-dependent manner while the concentration of SP went up. The Isc responses reached a peak value when the specimens were stimulated by 10^{-6} mol/L SP (Fig 1).

In order to examine whether the NK_1 receptor was involved in the enhancement of Isc when stimulated by SP, we observed the effect of NK_1 receptor antagonist CP-96345 pretreatment on the Isc responses to SP stimulation. The results showed that the NK_1 receptor antagonist CP-96345 at $>10^{-5}$ mol/L concentration inhibited the Isc responses to SP (10^{-6} mol/L) in nasal mucosal epithelial specimens from sen-

Fig 2. Isc changes of sensitized nasal mucosal epithelial tissues evoked by stimulation of SP at concentration of 10^{-6} mol/L after pretreatment with NK1 receptor antagonist CP-96345 at different concentrations: group E, 5×10^{-6} mol/L; group F, 10^{-5} mol/L; and group G, 5×10^{-4} mol/L. Control group rats were sensitized by toluene diisocyanate (TDI), and their nasal mucosal tissues were stimulated by 10^{-6} mol/L SP. Asterisk — $p < .05$ compared with controls.



sitized rats. These results provide support for the hypothesis that SP induces Isc responses by acting on NK1 receptors in rat nasal mucosa (Fig 2).

To observe the effects of neural inhibitors on Isc response was to test the possibility that SP can influence epithelial ion secretion indirectly, by promoting the release of neurotransmitters from sensory nerve endings. The results showed that TTX (10^{-6} mol/L) significantly inhibited the Isc in response to 10^{-6} mol/L SP. This result indicates that SP acts to induce secretion in rat nasal mucosa, in part via effects on sensory nerves (Fig 3).

Mast cells represent a major source of histamine in allergic rhinitis nasal mucosa, and this mediator is released on mast cell activation. Therefore, using the H1 and H2 antagonists pyrilamine and ranitidine, respectively, we evaluated the antagonism of histamine receptors that might influence the SP-induced Isc response. Pyrilamine and ranitidine significantly inhibited the SP-induced Isc responses; this finding suggests that H1 and H2 receptors are involved in the changes in Isc associated with treatment with SP. Moreover, pretreatment of nasal mucosal specimens with TTX and the H1 receptor antagonist pyrilamine induced a further reduction in the Isc responses to SP, compared with treatment with TTX or pyrilamine alone. These findings may reflect a direct effect of the neuropeptide on mast cells in the regulation of nasal mucosal secretion (Fig 3).

DISCUSSION

Previous studies showed that there was a dense network of SP nerve fibers in nasal mucosa, some of them located immediately under the epithelium.[7] Substance P is found in nasal mucosal nonmyelinated sensory nerve fibers, also called C fibers; it causes neurogenic inflammation through an axon reflex and mucosal hypersecretion, with rhinorrhea, congestion, and nasal obstruction.

Secretion of nasal mucosa is an active process that depends on ion secretion and absorption. The secreting ion is mainly chloride ion (Cl^-), and the natrium ion is the main absorption ion. Transepithelial movement of Cl^- includes a diffusion of Cl^- up its electrochemical gradient through the apical membrane to the surface of the epithelium. When the Cl^- secretion increases in nasal epithelium, the Isc goes up.[8] The results of the present study showed that Isc increased in response to SP stimulation in a dose-dependent manner. This means that SP is able to facilitate Cl^- secretion of nasal epithelial cells. This result is also consistent with the clinical features of allergic rhinitis: an increase in nasal mucosal hypersecretion, congestion, and nasal obstruction, and a higher content of SP in nasal secretions.[9]

Substance P can bind to all three of the known

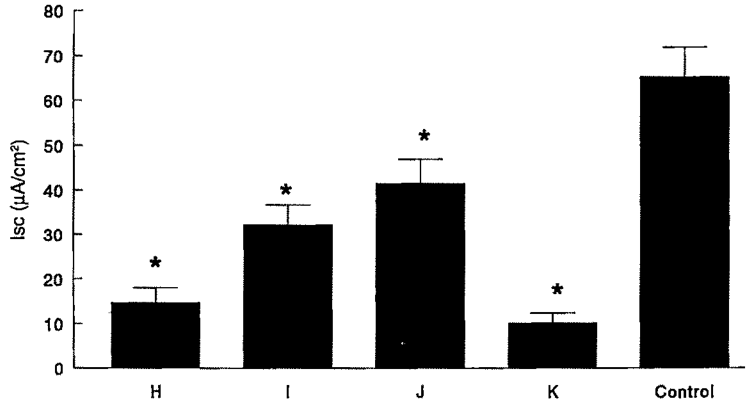


Fig 3. Isc changes of sensitized rat nasal mucosal tissue when stimulated with 10^{-6} mol/L SP after pretreatment with various antagonists and inhibitors: group H was pretreated with H1 receptor antagonist pyrilamine (5×10^{-6} mol/L), group I was pretreated with H2 receptor antagonist ranitidine (5×10^{-6} mol/L), group J was pretreated with neurotoxin tetrodotoxin (10^{-6} mol/L), and group K was pretreated with both pyrilamine (5×10^{-7} mol/L) and tetrodotoxin (10^{-6} mol/L). Animals of control group were also sensitized by TDI and stimulated by 10^{-6} mol/L SP. Asterisk — $p < .05$ compared with controls.

types of NK receptors, but has the highest affinity for NK1 receptors.[10] Studies with SP antagonists have suggested that SP-evoked Isc responses in guinea pig ileum are mediated by NK1 receptors.[11] Our results provide further support for this hypothesis. Pretreated with NK1 receptor antagonist CP-96345, an agent that has been found to be highly selective for the NK1 receptor in several species (rat, guinea pig, dog, and rabbit), the effect of SP stimulation on nasal mucosal Isc response was blocked significantly in a dose-dependent manner, and it was almost abolished by pretreatment with CP-96345 at the concentration of 5×10^{-4} mol/L. These results indicate that SP can evoke nasal mucosal epithelium ion secretion, and SP does act through NK1 receptors in rat nasal epithelium.

A morphologic association between mast cells and unmyelinated nerve fibers has been established. On electron microscopy, the sensory nerves located close to mast cells contain SP. On immunohistochemistry, histamine-containing mast cells and SP-positive nerve fibers are found in close association around blood vessels.[4] Johnson and Erdos[12] reported that SP causes histamine release from rat peritoneal mast cells. Histamine is one of the major chemical mediators in nasal allergy, and mast cells are the main source of histamine in nasal mucosa. Histamine plays an important role in the pathophysiology of allergic rhinitis.[13] This can be confirmed by the fact that treatment of allergic rhinitis with histamine receptor antagonists can dramatically stop the clinical syndrome.[14] It is reasonable to speculate there is a functional association between mast cells and SP-containing nerve endings. Our results indicate that the increase in Isc response to the stimulation of SP in nasal epithelium can be blocked partially by pretreatment with H1 receptor antagonist or H2 receptor antagonist. They suggest that the Isc response to SP in nasal epithelium may be partly, if not completely, due to activation of H1 and H2 receptors on nerve endings in the epithelial layer, which elicits them to release SP and subsequently activate the NK1 receptors on mast cell membrane to elicit mast cell degranulation. Of course, we have not directly investigated whether specific receptors for SP are expressed on rat nasal mucosal mast cells. However, we showed that in rat nasal mucosa, treatment with pyrilamine caused a further inhibition of the SP response. In other words, neurons blocked by TTX apparently could not completely prevent SP from releasing histamine. This finding, when taken together with the observation that pretreatment of nasal mucosal tissues with the specific NK1 receptor antagonist CP-96345 virtually abolished the Isc response to SP, provides indirect evidence for the presence of NK1 receptors on rat nasal mucosal tissues. However, SP may release certain mediators and/or cytokines from other NK1 receptor–bearing cells[15] that are resident in the nasal mucosa, and these chemicals may then act on mast cells. Thus, none of our findings rule out the possibility that SP can activate rat nasal mucosal mast cells via mechanisms other than an interaction with specific receptors on this cell type.

Taken together, our results indicate that SP plays an important role in sensitized rat nasal mucosal epithelial ion secretion. It may be by way of release of histamine from the mast cells resident in nasal mucosa. The results also suggest that NK1 receptor may mediate the effects of SP on nasal mucosal epithelial cell ion secretion, perhaps by NK1 receptor location on mast cells, sensory nerve endings, and epithelial cells.

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CARBON DIOXIDE LASER MICROSURGERY FOR EARLY SUPRAGLOTTIC CARCINOMA

PETRA AMBROSCH, MD
GOETTINGEN, GERMANY

MARTINA KRON, PHD
ULM, GERMANY

WOLFGANG STEINER, MD
GOETTINGEN, GERMANY

Forty-eight untreated patients with early supraglottic carcinoma (12 patients stage I and 36 patients stage II) had primary carbon dioxide laser microsurgery between 1979 and 1994 with the intent of complete tumor removal with preservation of functionally important structures of the larynx. Ninety-six percent of the patients were treated exclusively by surgery; 4% had laser microsurgery and postoperative radiotherapy. With a median follow-up of 55 months, the 5-year local control rate with the first treatment was 100% for pT1 cases and 89% for pT2 cases. The ultimate local control rate with voice preservation, including patients successfully salvaged after a local recurrence, was 97% for pT2 carcinomas. Five (10%) patients died of tumor (TNM)–related deaths. The 3-year recurrence-free rate and 3-year overall survival rate (Kaplan-Meier) were 87% and 85%; the 5-year recurrence-free rate and 5-year overall survival rate were 83% and 76%, respectively. The results achieved with transoral laser microsurgery in early supraglottic carcinoma are comparable to those of open supraglottic laryngectomy with respect to local control and survival. The functional results are superior, since clinically relevant aspiration did not occur.

KEY WORDS — carbon dioxide laser microsurgery, supraglottic squamous cell carcinoma.

INTRODUCTION

An early supraglottic carcinoma is one that has not metastasized to the regional lymph nodes and has not invaded the preepiglottic space or caused fixation of the vocal cord. Many laryngologists have shown that supraglottic laryngectomy is an effective therapeutic approach for these lesions. Alternatively, early supraglottic carcinomas may be treated successfully with radiotherapy. Since the supraglottic laryngectomy adds significant morbidity to the patient and some patients have to be excluded from surgery due to anticipated postoperative functional disorders, the less invasive carbon dioxide (CO_2) laser excision, alone or combined with radiotherapy, came into use. Vaughan[1] first described CO_2 laser resection of supraglottic cancer in 1978. Davis et al,[2] in 1983, discussed the first series of 20 patients undergoing laser epiglottectomy for removal of obstructing benign epiglottic lesions and limited suprahyoid epiglottic cancer. Subsequently, Davis et al[3] and Zeitels et al[4] reported several series of CO_2 laser supraglottic cancer resections in selected cases. In 1979, Steiner et al[5-7] began to employ less invasive laser microsurgical resection, instead of open supraglottic laryngectomy, for the curative treatment of supraglottic carcinomas.

The aim of this article is to present the results of primary laser microsurgical treatment to the primary tumor combined with selective neck dissection in early supraglottic carcinoma and to compare these data with those obtained with classic conservation surgery and primary radiotherapy.

PATIENTS AND METHODS

Patients. A total of 44 patients were treated for early (stage I and II) squamous cell carcinoma of the supraglottic larynx from August 1986 to December 1994 at the Department of Otorhinolaryngology–Head and Neck Surgery of the University of Goettingen, Germany. All patients were treated with transoral laser microsurgery on the primary tumor; no patient received open supraglottic laryngectomy or primary radiotherapy.

Ten patients were excluded from the analysis: 2 patients who were previously treated elsewhere, and 7 patients who had previous other malignant tumors. Further excluded was 1 patient who died the night after surgery; autopsy failed to reveal the cause of death. To the remaining 34 patients were added another 14 patients treated between 1979 and May 1986 at the Department of Otorhinolaryngology–Head and Neck Surgery of the University of Erlangen, Germany (surgeon W.S.), so that this report comprises a total of 48 patients.

From the Department of Otorhinolaryngology–Head and Neck Surgery, Georg-August-University of Goettingen, Goettingen (Ambrosch, Steiner), and the Department of Biometry and Medical Documentation, University of Ulm, Ulm (Kron), Germany. This work was supported by a grant from the Deutsche Krebshilfe, Dr Mildred Scheel Stiftung (M 18/95/Am3).

CORRESPONDENCE — Petra Ambrosch, MD, Dept of Otorhinolaryngology–Head and Neck Surgery, University of Goettingen, Robert-Koch-Str. 40, D-37075 Goettingen, Germany.

The patients ranged in age from 39 to 82 years (median age 61 years). Seventeen (35.4%) were over 65 years of age. Forty-two were men and 6 were women.

Preoperative Examination. The routine preoperative examination comprised magnifying laryngoscopy, palpation, and, since 1989, ultrasonography of the neck nodes. Computed tomography (CT) was not routinely employed. For the exclusion of possible concomitant second primary tumors and distant metastases, bronchoscopy, esophagoscopy, radiography of the chest, and ultrasonography of the abdomen were performed. Panendoscopy was done together with the resection of the primary tumor. Pulmonary function tests (vital capacity and forced expiratory volume in 1 second) were performed only in patients with a history of chronic obstructive pulmonary disease. The indication for laser microsurgical resection of supraglottic carcinoma was not influenced by the results of the pulmonary function tests.

Operative Technique. Optimal exposure and visualization of the supraglottic region is decisive for a safe tumor resection. The operations were carried out with a Heraeus Laser Sonics LS 500 laser coupled to the operating microscope. The focus diameter of the micromanipulator was 0.5 mm, and the delivered laser energy was about 2,080 to 3,900 W/cm^2. The carbon dioxide laser coagulates vessels with a maximum diameter of 0.5 to 1 mm. Bleeding from larger branches of the superior laryngeal vasculature was controlled by electrocautery. Only exceptionally large vessels like the superior laryngeal artery were clipped. Since the early 1980s, we have used a bivalved laryngopharyngoscope (Steiner's adjustable laryngopharyngoscope) for transoral laser microsurgery in the supraglottic larynx, base of the tongue, and hypopharynx. With the lower blade, the endotracheal tube is pushed against the posterior pharyngeal wall. The superior blade is placed in the glossoepiglottic vallecula. After positioning of the laryngoscope, the blades are separated. Multiple changes of the position of the laryngoscope are necessary to achieve good visualization of the tumor and the surrounding healthy tissue during the entire operation. Only small carcinomas of the free edge of the suprahyoid epiglottis or the ventricular fold are amenable to en bloc resection as an excisional biopsy. In case of more extended lesions, the resection is done piecemeal. We usually start with sagittal splitting of the epiglottis in the midline. The suprahyoid parts of the epiglottis are removed. The infrahyoid epiglottis is sagittally split and the preepiglottic fat is divided until the thyroid cartilage is visualized. Gross infiltration of the preepiglottic fat can be seen under the microscope. Microscopic invasion, which is common in tumors of this location, is detectable by histopathologic evaluation of the resected specimen only. Because of possible infiltration of the preepiglottic space, we recommend resection of the preepiglottic fat at least subtotally in cases of infrahyoid epiglottic involvement. In T2 cases, the resection is extended to one or both false vocal cords. In cases of tumor extension to the paraglottic space, the tumor is followed and parts of the vocalis muscle are resected.

The resected specimens were histopathologically examined by step serial sections.[8] If histopathologic analysis showed a positive resection margin, a re-resection was carried out to obtain clear margins. In cases of exposed thyroid cartilage, perioperative antibiotic prophylaxis, usually with penicillin, was performed.

Treatment of Primary Tumor. All patients received transoral laser microsurgical resection of the supraglottic carcinoma. Thirty-two patients had a transoral laser microsurgical tumor resection comparable to standard supraglottic partial resection, including the epiglottis, the preepiglottic fat, and both false vocal cords, but preserving the thyroid cartilage. In 4 of these patients the resection was extended to the paraglottic space. Sixteen patients had less than this procedure, eg, epiglottectomy or partial epiglottectomy, or epiglottectomy combined with the resection of one ventricular fold.

Pathologic staging according to the 1992 UICC classification[9] was used for the primary tumor and the neck. Twelve patients had a tumor categorized pT1. Cancer involving the epiglottis accounted for 6, and cancer involving one false cord for another 6 cases. Thirty-six patients had pT2 lesions. Cancer involving the epiglottis and one false cord accounted for 24 and cancer involving the epiglottis and both false cords accounted for 12 of the pT2 cases.

Six patients (12.5%) received a re-resection because of positive resection margins. In 5 cases the re-resection specimens were free of cancer; in 1 case residual cancer was found. This patient was treated with another two re-resections until he was free of tumor. Another 8 patients (17%) had microlaryngoscopic removal of granulation tissue. In 7 cases, the clinical diagnosis was proven by histology, and in 1 case, residual carcinoma was found (R1 resection). This patient refused further surgery and was subjected to postoperative radiotherapy.

Treatment of Neck. Twenty-six patients received either a unilateral (11 patients, 23%) or a synchronous bilateral (15 patients, 31%) neck dissection. Of the latter patients, all had primary tumors extending across the midline. Twenty-two (46%) patients had

TABLE 1. ANALYSIS OF LOCAL RECURRENCES

TNM	*Time Since Surgery (mo)*	*Site of Recurrence*	*Treatment*	*Outcome*
pT2N0	4	Laryngeal remnant (rT2)	Open supraglottic laryngectomy	Free of disease 135 months after salvage surgery
pT2N0	6	Laryngeal remnant (rT2)	Laser microsurgery	Free of disease 41 months after salvage surgery, died of second primary tumor (primary liver cancer)
pT2pN0	16	Laryngeal remnant (rT4)	Laser microsurgery	Free of disease 33 months after salvage surgery
pT2N0	52	Laryngeal remnant, hypopharynx (rT4)	Refused total laryngectomy	Died of local disease

no neck dissection. The neck dissections were done simultaneously with the primary treatment in 8 cases and delayed in 18 cases. The time delay incurred had a mean interval of 12 days (range 5 to 50 days). In all cases, selective neck dissections with removal of the lymph nodes of levels II and III were carried out.

Postoperative Radiotherapy. Two (4%) patients underwent postoperative radiotherapy of the primary site and the regional lymphatics. In 1 patient a re-resection proved microscopic residual cancer, as mentioned above, and in the other case the rationale for postoperative radiotherapy remains unclear in retrospect.

Statistical Methods. Follow-up data were available on all patients. Each of them was followed until death for those who died or until December 1996 for those who were alive. The median follow-up period was 55 months (range 2.5 to 163 months). Survival calculations were done by the Kaplan-Meier method.[10] The overall survival time was defined as the interval between the date of surgery and the date of the last consultation, or date of death. For the determination of recurrence-free survival, intercurrent deaths and deaths due to second primary tumors, as well as patients alive without recurrence, were regarded as censored observations. Uncensored observations included local and neck recurrences, distant metastasis, and TNM-related deaths.

RESULTS

Local Control. Four of the 48 patients (8%) developed local recurrences. In all cases, the laryngeal remnant was the site of recurrence. Three patients had secondary treatment to the primary site and were successfully salvaged: 2 patients were treated by further transoral laser microsurgical resection of the recurrence, and 1 patient by open supraglottic laryngectomy. One patient refused further surgery as well as radiotherapy and was treated palliatively. No salvage laryngectomy was performed.

The 5-year local control rate was 12 of 12 (100%) for stage I (pT1N0/pN0) and 32 of 36 (89%) for stage II (pT2N0/pN0) cases. The ultimate local control rate with voice preservation, including patients successfully salvaged after a local recurrence, was 35 of 36 (97%) for stage II carcinomas. The analysis of local recurrences, salvage therapy, and outcome is shown in Table 1.

Regional Control. Of the 26 patients who had unilateral dissection, 1 developed a late metastasis (rN2a) in the dissected side in the supraclavicular fossa (level V). The 15 patients who had bilateral neck dissection remained free of recurrences. Of the 22 patients who had no neck dissection, 1 developed a late metastasis (rN3) in the upper jugular nodes (level II) of the neck ipsilateral to the primary tumor. Both patients with neck recurrences were treated by radical neck dissection and postoperative radiotherapy. One patient died of the neck metastasis, and the other with neck metastasis due to distant metastases in the lung. The analysis of neck recurrences, salvage therapy, and outcome is shown in Table 2.

TABLE 2. ANALYSIS OF LATE METASTASES

TNM	*Time Since Surgery (mo)*	*Site of Recurrence*	*Neck Dissection**	*Treatment*	*Outcome*
pT2N0	11	Nondissected neck† (rN3)	None	Neck dissection and radiotherapy	Died of neck recurrence
pT2pN0	16	Dissected side (rN2a)	Unilateral	Neck dissection and radiotherapy	Died of distant metastases with neck recurrence

*Done at time of initial treatment.

†Occurred in nondissected neck ipsilateral to primary tumor.

TABLE 3. CAUSES OF DEATH

Cause of Death	*No. of Patients*	*%*
TNM-related death	5	10
Local recurrence	1	2
Neck recurrence	1	2
Distant metastases	3	6
Second primary tumor	2	4
Intercurrent disease	8	17
Unknown	1	2

TABLE 4. SURVIVAL RESULTS

	Years	*Survival Rate (%)*	*95% Confidence Interval*
Overall survival	2	89	80, 98
	3	85	74, 95
	5	76	63, 89
Recurrence-free survival	2	87	77, 97
	3	87	77, 97
	5	83	72, 95

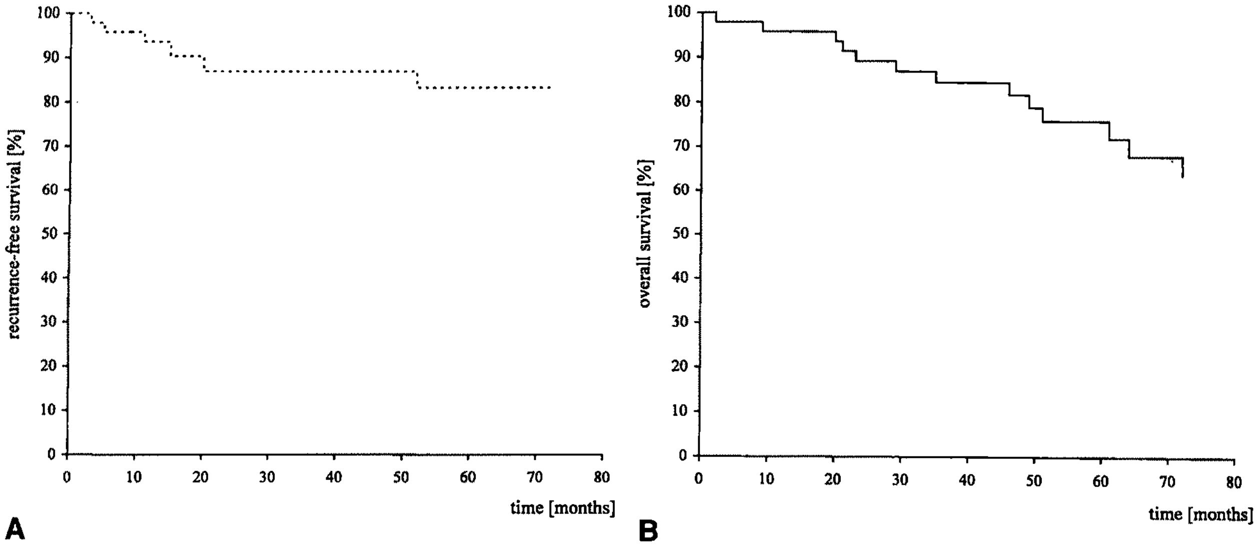
Distant Metastases. Two (4%) patients developed distant metastases without local or neck recurrence. One of them had bone metastases 13 months after primary treatment, and the other, mediastinal metastases 95 months after primary treatment, without diagnosis of a second primary carcinoma.

Second Primary Tumors. Second primary malignancies occurred in 5 (10%) patients. In 1 case the second primary cancer was located in the head and neck region (oral cavity). In 3 cases the second primary tumors occurred in other locations (esophagus, lung, liver); in 1 case a non-Hodgkin's lymphoma was observed.

Survival. The causes of death (follow-up until December 1996) are listed in Table 3. The recurrence-free and overall 3-year survival rates were 87% and 85%, respectively. The corresponding 5-year survival rates were 83% and 76%, respectively (Table 4). The Kaplan-Meier estimates of recurrence-free and overall survival are shown in the Figure.

Postoperative Complications. Major complications were defined as complications of treatment requiring surgical management. Two (4%) patients experienced postoperative endolaryngeal hemorrhage. In both cases the bleeding could be managed by further microlaryngoscopy and endoscopic electrocoagulation. All wounds healed by secondary epithelialization, and there were no problems with perichondritis or chondritis, despite the majority of wound cavities having exposed thyroid cartilage. One patient developed pneumonia in the immediate postoperative period. Late complications did not occur. No patient had been tracheotomized together with the resection of the primary tumor. Thirty-five (73%) patients were extubated immediately after laser surgery. Thirteen (27%) patients had prolonged intubation (extubation within 24 hours in 10 patients, and extubation in 24 hours or more in 3 patients).

Functional Results. The median duration of nasogastric feeding was 5 days (range 3 to 30 days; mean duration 6 days). One patient was tracheotomized after a resection extending to the paraglottic space and the arytenoid region bilaterally to prevent aspiration pneumonia. After 30 days the feeding tube was removed, and after 8 weeks the laryngeal protective function was normal and the patient was decannulated. He is free of disease 158 months after surgery without pulmonary complications. The other patients achieved oral intake after removal of the feeding



Kaplan-Meier estimates of A) recurrence-free and B) overall survival after laser microsurgical supraglottic resection (stage I and II, n = 48 patients).

tube. No patient received swallowing therapy. There was no secondary laryngectomy for functional reasons. One patient, who had a tumor resection extending to the paraglottic space bilaterally down to the inferior border of the thyroid cartilage, developed vestibular stenosis necessitating endoscopic laser resection of the supraglottic scar tissue.

DISCUSSION

The treatment of early supraglottic carcinoma is still a controversial, unsettled issue. There are two treatment choices for stage I and II carcinoma of the supraglottis: surgery or radiotherapy used as single agents. In recent years, some reports have been published on experience with laser surgery. The most important measures of success of a treatment form are survival, primary control with the first treatment, neck control, and the percentage of patients with glottic voices.

Survival. The recurrence-free and the overall 3-year survival rates were 87% and 85% in our series of laser microsurgically treated early supraglottic carcinomas. The 5-year recurrence-free and overall survival rates were 83% and 76%. These figures are comparable to those reported in other surgical series. Several authors have reported 3-year corrected survival rates of between 67% and 92% for stage I disease and between 80% and 85% for stage II disease.[11-16] The corrected survival rates at 3 to 5 years of stage I and stage II supraglottic carcinoma treated with irradiation alone were reported as 69% to 100% and 74% to 90%, respectively.[17-21]

As expected with early supraglottic carcinoma, most patients in our series (17%) died of causes other than their cancer. Five (10%) patients died of TNM-related deaths; among them, 3 of distant metastases. In 1 of the cases the distant metastases were diagnosed 95 months after primary treatment, and an occult second primary tumor seems very likely.

Local Control. In our series, the 5-year local control rate with the first treatment was 100% for pT1 and 89% for pT2 cases. Frequent postoperative examinations by the operating surgeons resulted in early detection of laryngeal recurrences and successful salvage in 3 of 4 cases. The ultimate local control rate with voice preservation was 97% for pT2 carcinomas.

Zeitels et al,[22] in a multi-institutional study, reported on 42 patients with endoscopically treated supraglottic cancer. Nineteen patients received en bloc resection of a T1 or T2 carcinoma without postoperative radiotherapy. Local control was 100% at 2 years; 1 patient developed neck recurrence and was treated by neck dissection and radiotherapy. The remaining 23 patients, all with T2 or T3 cancer and N0 neck, had endoscopic resection and postoperative radiotherapy to the primary and the neck. Four of these patients had local recurrences and were laryngectomized. One of the 4 laryngectomy patients died of local disease, and 3 patients died of neck recurrence. In the aforementioned group, the authors regard endoscopic laser resection as "neoadjuvant therapy with a histopathologically controlled result." Eckel and Thumfart[23] reported 15 patients with laser resected T1 and T2 supraglottic carcinoma. With an average follow-up of 22 months, all patients were free of local recurrences; 1 patient developed a neck recurrence. Rudert and Werner[24] treated 30 patients with supraglottic carcinoma of all T categories endoscopically with curative intent. In 21 cases, postoperative radiotherapy was given. Among 15 cases with T1 and T2 disease, 1 local recurrence and 1 TNM-related death were recorded.

Horizontal supraglottic laryngectomy alone controls T1 carcinoma at a rate of 90% to 100%, and T2 carcinomas are controlled at 80% to 97%.[11-14,25-29] Bocca[27] reported a series of 537 patients treated with supraglottic laryngectomy. The proportion of patients with local control was 94% for stage I (44 of 47 patients) and 82% for stage II cases (206 of 252 patients). DeSanto[12] reported 70 patients who underwent supraglottic laryngectomy for pathologic stage I (26 patients) and stage II disease (44 patients); no primary recurrences were observed. Suárez et al[14] reported 193 patients treated with supraglottic laryngectomy. The local control rates were 98% (40 of 41 patients) for T1 and 91% (98 of 108 patients) for T2 cases. Herranz-Gonzáles et al[29] presented a series of 110 patients with supraglottic laryngectomy. The local control 3 years after surgery was 92% for stage I (23 of 25 patients) and 97% for stage II disease (36 of 37 patients). Martinez et al[15] reported after supraglottic laryngectomy a local recurrence rate of 10% (35 of 347 patients) for T1 cases and of 6.4% (17 of 266 patients) for T2 cases.

Primary radiotherapy controls T1 carcinomas at 77% to 100% and controls T2 carcinomas at 62% to 83%.[17,20,21,30-34] While Inoue et al[20] found a significantly better local control rate for tumors of the epilarynx than for tumors of the lower supraglottis, others could not find a difference in local control for different subsites of the supraglottis.[34,35] In case of recurrence, patients initially eligible for a supraglottic laryngectomy before irradiation most often require a total laryngectomy. Johansen et al[18] reported 117 patients with early supraglottic carcinomas treated between 1963 and 1985 with primary radiotherapy. Forty-eight percent of the patients experienced recurrences and 31% underwent salvage laryngectomy. Inoue et al[20] treated 100 patients with T1N0 and T2N0 supraglottic carcinoma with primary radiotherapy. The 5-year local control rates of T1 cases were 77%, and of T2 cases, 62%. Seventeen percent of the patients were laryngectomized. Mendenhall et al[21] reported with radiotherapy alone a 5-year local control rate of 100% for T1 and 83% for T2 cases. However, 20% of the patients had been excluded because of death from other causes. Fourteen percent of the patients with T2 lesions required total laryngectomy for salvage.

Two patients in our series had adjuvant radiotherapy. One patient had positive resection margins of a re-resection and refused further endoscopic treatment or an open surgical approach. In our opinion, it is a dangerous policy to rely on postoperative full course radiotherapy to eradicate microscopic disease, but we were forced to make a compromise in this case. In general, we prefer repeated re-resections with the aim of obtaining clear margins. In the other patient the treatment decision remains unclear in retrospect. We find no need for combined therapy for patients with early disease. Some authors recommend transoral laser resection followed by postoperative radiotherapy in selected cases, especially in patients with larger tumors who refuse open surgery and in whom there is a high probability of local failure with radiotherapy alone.[22,36]

Regional Control. Both sides of the neck are at risk for harboring occult metastases, and generally neck failure is recognized as a major problem in the treatment of supraglottic carcinoma. Especially, the appropriate treatment of the clinically N0 neck is still controversially discussed. Some authors argue for routine bilateral neck dissection[13,26-28,37,38] or for elective irradiation of the neck in all patients.[39] Other authors suggest that contralateral elective neck dissection is not required in negative unilateral neck dissections[12,40] or in lateralized primary tumors.[41,42] The use of frozen sections from one side of the neck as a key for the dissection of the second side, as proposed by DeSanto,[12] is an attractive option practiced by us as well.

The type of neck treatment selected by us depends on several variables, including (p)T category, maximum depth of invasion of the primary tumor, clinical neck stage, patient age, and general health. The patients (46%) who had no neck dissections were on average 6 years older than patients who had them. In 54% of the patients, unilateral (23%) or bilateral (31%) selective neck dissections limited to levels II and III were carried out. In agreement with other authors,[38,42] we did not find an increase in morbidity due to bilateral selective neck dissection. In our study, 2 neck recurrences (4%) occurred, and both were fatal. One patient who did not have initial neck therapy required a delayed neck dissection and died of cancer; 1 patient developed a late neck metastasis following unilateral neck dissection. Contralateral late metastases did not occur. The rate of late neck metastases in our study is comparable to those of other surgical series.

In the study by Lutz et al,[13] the rate of late metastases was 13% (14 of 107 patients) when no neck metastases were identified in the dissected neck. A subsequent study by the same group[28] on the use of routine bilateral neck dissection in supraglottic cancer demonstrated a 5% neck recurrence rate in N0 cases (2 of 38 cases). Suárez et al[14] observed, regardless of T-stage, an 11.5% neck recurrence rate in pN0 cases (14 of 121 cases). In a previous study, the authors found in N0 cases a recurrence rate of 2% in the dissected side and of 13% in the undissected side. In untreated necks, a relapse rate of 18% was found.[37] The authors therefore conclude that patients with supraglottic carcinoma should receive bilateral neck dissection. In a study of all primary stages carried out by DeSanto et al,[40] there was only 1 delayed contralateral metastasis in 98 patients who had unilateral negative dissections. Routine bilateral neck dissection in early supraglottic cancer, although not associated with a significantly higher morbidity in our series, does not appear to be justified by our data.

Complications. Another parameter of treatment success is the complication rate. In our series 4% of the patients experienced postoperative hemorrhage, which was controlled by endoscopic coagulation. Endolaryngeal bleeding in endoscopically treated, nontracheotomized patients is a serious complication, not only because of the blood loss, but because of possible aspiration of blood. The reported incidences of postoperative bleeding after open supraglottic laryngectomy vary between 1.6% and 5%.[29,43,44] The relatively high percentage of postoperative hemorrhage after endoscopic resection might be due to the sole use of cautery to stop bleeding from the superior laryngeal vessels. We hope that clipping of bigger vessels will have advantages over cautery and diminish the complication rate in the future.

In 1 case with near-complete resection of the paraglottic muscles, vestibular stenosis occurred after laser microsurgery. Staffieri[45] reports a 5% incidence of vestibular stenosis following standard supraglottic laryngectomy, and Robbins et al[25] noted a 5.8% incidence of permanent tracheotomies. Krespi and Khetarpal,[46] in a literature review, quantified the incidence of persistent laryngeal edema and stenosis, with delayed decannulation or late recannulation, as varying from 2% to 15% after horizontal partial laryngectomy with or without irradiation. With laser microsurgery the incidence of postoperative stenosis was low. The reason might be that factors that cause excess scar formation, such as loss of cartilage, wound infection, perichondritis, and chondronecrosis, were absent.

Functional Results. It is well known that most patients undergoing supraglottic laryngectomy aspirate to some extent, but the frequency and severity have varied considerably in previous reports. Rademaker et al[47] studied the recovery of postoperative swallowing function after various partial laryngectomy procedures. Among 20 patients with nonextended supraglottic laryngectomies, oral intake of food was achieved after a median time of 26 days. The feeding tube was removed after a median time of 40 days, and the median time to attainment of preoperative diet was 91 days. Hirano et al[48] reported that 32 (84%) of 38 patients who had supraglottic laryngectomy were weaned from tube feeding within 30 days, and 3 patients required 34 to 61 days prior to resuming oral intake. The remaining 3 patients, who had extended procedures with removal of one arytenoid cartilage, failed to reestablish oral nutrition and needed completion laryngectomy. Flores et al[49] divided 31 patients with supraglottic laryngectomy and preservation of both arytenoids into three groups according to deglutition problems: 71% were in the "successful" category (no or clinically insignificant aspiration, removal of nasogastric tube within 20 days), 23% "delayed" (prolonged nasogastric tube feeding from 20 days to 5 months), and 6% "failed" (intractable aspiration, placement of a gastrostomy tube, completion laryngectomy). Beckhardt et al,[50] using the criteria of Flores et al to classify 46 patients (29 standard supraglottic laryngectomies, 17 extended procedures), found that 61% were "successful," 24% "delayed," and 15% "failed." The authors could not prove statistically significant differences for base of tongue and arytenoid resection. Staffieri[45] reported that in a series of 143 supraglottic laryngectomies, the feeding tube was removed after an average of 17 days (range 6 to 57 days). Three (2%) patients were never decannulated, due to aspiration. Bocca[51] reported delayed swallowing recovery (nasogastric feeding for several weeks) in 5.4% of 240 patients; 1 patient had completion laryngectomy due to aspiration.

Videofluorographic studies have been carried out to examine pharyngeal and laryngeal movement during deglutition after supraglottic laryngectomy.[52-54] The primary means of laryngeal protection seems to be the apposition of approximated arytenoids to the epiglottic base that normally occurs in conjunction with hyolaryngeal anterior-superior displacement. Logemann et al[53] identified two critical factors in recovery of swallowing in supraglottic laryngectomy patients: airway closure at the laryngeal entrance (space between the arytenoid cartilage and base of tongue) and the movement of the tongue base to make complete contact with the posterior pharyngeal wall. They observed that when patients achieved these two functions, they returned to normal swallowing. The incidence of aspiration seems to vary with the patients' age and general condition and the extent of arytenoid and base of tongue resection. Other factors believed to contribute to aspiration are paralyses of laryngeal and pharyngeal branches of the vagus nerve.

The functional results after transoral laser microsurgery, with a median duration of nasogastric feeding of 5 days and only 1 case of delayed recovery necessitating temporary tracheotomy to prevent aspiration pneumonia, are very favorable. We attribute the very early functional recovery to the integrity of the tongue base and pharyngeal musculature (permitting a normal pattern of mobility), the preservation of mobile arytenoid cartilages, and the preservation of the hyoid bone and the strap muscles, which provide the normal anatomic suspension of the larynx. Further, the extralaryngeal parts of the superior laryngeal nerves are preserved, contributing to restitution of the sensory function.

CONCLUSION

We can conclude that the results achieved with transoral laser microsurgery in early supraglottic carcinoma are comparable to those of open supraglottic laryngectomy with respect to local control and survival. They are slightly better than the results reported with primary radiotherapy with respect to local control and survival, and much better regarding the number of glottic voices preserved. The incidence of postoperative complications was lower than after conventional surgery. A meticulous surgical technique to avoid postoperative bleeding and mucosal edema is of importance. Since laser microsurgery allows a resection exactly adapted to tumor spread, it is possible to preserve functionally important structures, thus avoiding disturbance of pharyngeal and laryngeal movement and permitting earlier and better swallowing rehabilitation. The spectrum of surgical treatment in early supraglottic carcinoma has been expanded by the addition of transoral laser microsurgery. The low postoperative morbidity and the good functional results allow us to expand the indications for supraglottic partial resection, especially in patients who previously were excluded from surgery for medical reasons. Our experience with laser microsurgery of advanced supraglottic lesions will be reported separately.

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IMAGE ANALYSIS OF BENIGN AND MALIGNANT NECK MASSES

RANDALL L. PLANT, MD, MS
NORFOLK, VIRGINIA

Current techniques for differentiating benign and malignant neck masses in computed tomography scans rely on basic features such as size and image density. Advances in digital imaging have led to development of sophisticated computerized image processing tools. Four different image processing algorithms were used to compare 10 patients with neck masses positive for squamous cell carcinoma and 11 patients with benign neck nodes. Statistically significant differences were seen between the malignant and benign masses for edge analysis and Fourier analysis. Significant differences were also seen in several texture parameters, although these results were considered less reliable. There were no differences between the two groups when compared by histogram analysis. It is concluded that both edge analysis and Fourier analysis can provide additional information to distinguish benign and malignant neck masses.

KEY WORDS — computer-assisted image processing, head and neck neoplasms.

INTRODUCTION

Cervical lymph nodes are one of the earliest sites for spread of head and neck squamous cell carcinoma (SCCA). The presence or absence of cervical metastases strongly influences the course of treatment and a patient's prognosis. The decision to irradiate a neck or perform a neck dissection frequently depends on the probability that cancer has spread to regional lymph nodes. Lymphadenopathy can often be detected with careful clinical examination. However, clinically occult nodes may be present deep to the sternocleidomastoid muscle or elsewhere in the neck. Benign reactive processes can also give rise to enlarged lymph nodes. The clinician must both identify nodal disease in the neck and determine whether this disease represents a malignant or benign process.

Fine needle aspiration is useful in confirming the presence of malignancy within an enlarged node. However, aspiration of a deep neck node is technically difficult and, if negative, provides little confirmatory information.

Imaging technologies have gained importance in detection of neck masses. Recent work has focused on using computed tomography (CT) scans to differentiate malignant and benign nodes. To aid in this differentiation, the clinician relies on certain image features characteristic of malignant or benign masses.

In early studies assessing CT scans of neck lymph nodes, Mancuso et al[1,2] used retrospective and prospective analysis to diagnose metastatic disease. Their criteria for malignant disease were 1) a discrete nonenhancing mass in lymph-bearing tissue greater than 15 mm in diameter; 2) a poorly defined or irregularly bordered mass; 3) grouping of three or more of contiguous 8- to 15-mm-diameter nodes; 4) a mass with a diameter of 15 mm and a low-density center; and 5) obliteration of tissue planes in lymph-bearing tissue.

A more recent study by van den Brekel et al[3] looked carefully at the influence of node size on the sensitivity and specificity of CT prediction of malignancy. Instead of overall diameter, they found greater accuracy using a minimal axial diameter measurement of 10 mm (11 mm in the subdigastric region). Using this size criterion, as well as the previously described criteria of central low density and fat plane obliteration, they found an overall sensitivity of 87% and specificity of 94% per neck specimen.

Som[4,5] has given a thorough review of the merits of CT and magnetic resonance imaging (MRI) in the differential diagnosis of cervical lymphadenopathy. Decrease in central nodal attenuation, indicative of central necrosis, was considered the most accurate CT criterion for presence of metastatic lymphadenopathy. Presence of poorly defined nodal margins was considered suspicious of extracapsular spread. Nodal size criteria for malignancy in this review were 15 mm for jugulodigastric and submandibular nodes, and 10 mm for all other nodes. Nodes exceeding this diameter were positive in 80% of the cases. Magnetic resonance imaging did not offer any specific advantage over CT scans, although new advances in coil design and pulse sequencing may improve MRI accuracy.

From the Department of Otolaryngology–Head and Neck Surgery, Eastern Virginia Medical School, Norfolk, Virginia.

CORRESPONDENCE — Randall L. Plant, MD, MS, Dept of Otolaryngology–Head and Neck Surgery, Eastern Virginia Medical School, 825 Fairfax, Fifth Floor, Norfolk, VA 23507-1912.

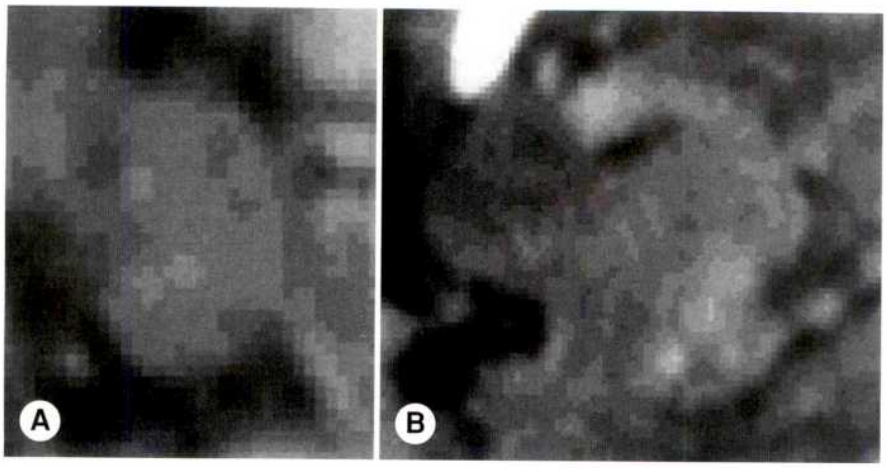

Fig 1. Computed tomography scans (enlarged) of A) benign node and B) malignant node.

Work to date on head and neck masses has primarily involved basic image criteria such as size and subjective evaluation of tissue density and contrast. There are many other image processing techniques that can be used to characterize and distinguish medical images. Kilday et al[6] combined seven shape descriptors and patient age to distinguish between breast cysts, cancers, and fibroadenomas. These descriptors only contained information about the outline of the object, and were not influenced by the lesion's internal structure. Linear discriminant analysis was used to separate the three classes of tumors. A neural network model used by Wu et al[7] was successful in distinguishing benign and malignant breast masses. Their three-layer network was based on 43 image features used by radiologists to classify breast lesions.

Computed tomography scans are initially acquired as digital data sets, and later converted to analog images on radiographic films. The intensity or brightness of each pixel corresponds to the density of tissue or bone at that point in the body. For most commercial scanners, data are collected at 12-bit resolution, ie, 4,096 gray scale levels. Since the eye can only distinguish approximately 50 different gray scale levels, a small segment of the entire 12-bit density range is displayed at one time. The original data set is adjusted to maximize contrast changes at the tissue density under question. For lymph node assessment, the scaling is centered around the soft tissue densities; for bone erosion, the window is centered around bone densities.

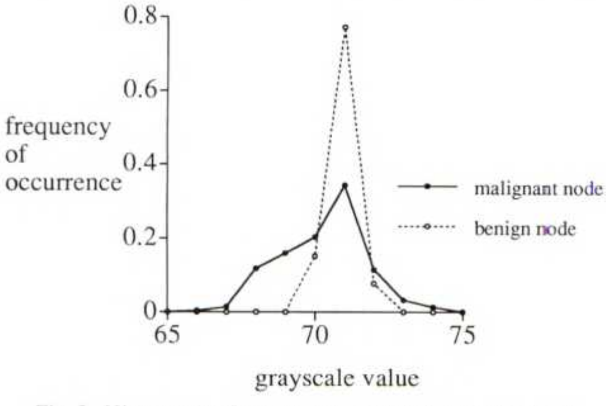

Fig 2. Histogram of gray scale values for benign and malignant nodes.

In addition to its visual pattern, an image can also be considered as a numeric matrix of gray scale pixel values. In digital image processing, a variety of mathematic calculations are performed on these gray scale values in order to enhance or to analyze the image. Many of the transformations duplicate, in a simplified fashion, similar processing done by the mind and the eye. However, digital techniques differ in that they can quantify image features and they can be performed simultaneously on the entire 12-bit data set.

The purpose of this report is to investigate whether digital signal processing techniques can aid in the diagnosis of neck masses. Four different image processing techniques were used: histogram analysis, edge analysis, texture analysis, and Fourier analysis.

Histogram Analysis. Histogram analysis quantifies the variability of the gray scale intensity of an image. The frequency distribution of the gray scale values is calculated and displayed as a histogram. If an image has low contrast, most pixels will have similar gray scale values and the histogram will have a narrow peak. An image with high contrast will have greater variation in gray scale values and therefore a broader and shorter peak.

Examples of histogram analysis are shown below. Figure 1 shows CT images of a benign and a malignant node. Figure 2 shows the gray scale histograms for these two masses. The central portion of the benign node has a uniform density, and its histogram is therefore a sharp peak centered about this gray

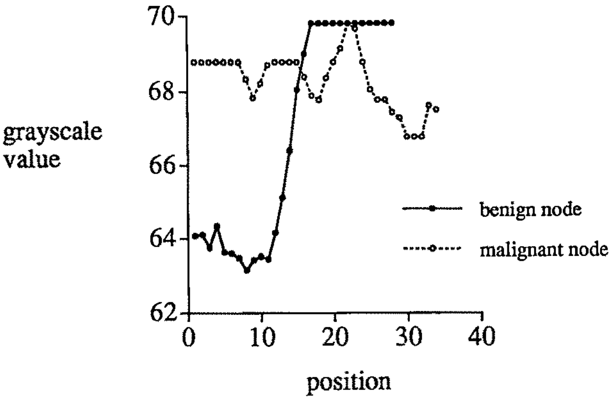

Fig 3. Edge response for benign and malignant nodes.

scale value. The malignant mass, in contrast, has greater density variation due to regions of central necrosis and tissue inhomogeneity. Its histogram is therefore broader and more shallow.

The amount of spread in each histogram can be calculated[8] based on moment calculations, where the ith moment mi is equal to

$$mi = \sum_{x=1}^{L} x^i\,(p(x))$$

where L = number of gray scale values and p(x) = probability density for gray scale value x.

The first moment, m1, is the mean, or weighted-average, gray scale. Central moments, μi, are defined in terms of the difference between a random variable and its mean value[9]:

$$\mu i = \sum_{x=1}^{L} (x - m1)^i\,(p(x))$$

The first central moment is zero, by definition. If we substitute |x − m1| for the term in parentheses, we obtain the absolute first central moment, μ1, also known as the dispersion:

$$\mu 1 = \sum_{x=1}^{L} (|x - m1|)\,(p(x))$$

The absolute second central moment, μ2, is the variance of the function:

$$\mu 2 = \sum_{x=1}^{L} (x - m1)^2\,(p(x))$$

For an image with low contrast, most of the pixels will have similar gray scale intensity. The difference between the mean pixel intensity and each individual pixel ($xi - m1$) will be small, resulting in low values for the dispersion and variance. As the variation in gray scales within the image increases, a corresponding increase will occur in the dispersion and variance. Because of the squared term used in its calculation, the variance is very sensitive to large differences from the mean pixel intensity.

The hypothesis tested in this study is that malignant masses will have greater variation in pixel intensity than benign nodes, and the dispersion and variance of their histogram will therefore be greater.

Edge Analysis. Edge analysis is a form of image processing that looks at the change in gray scale intensity along a line across regions of the image. If the region of interest is uniform in gray scale intensity, the edge response (also called the profile) will be quite flat. However, if there are large changes in contrast along the selected line, the profile of the gray scale will fluctuate in intensity. In going from a light region (high gray scale intensity) to a dark region (low gray scale intensity), the profile consists of a step function. The slope of the step depends on the sharpness of the transition from the light to the dark region. Differentiation of the step function produces a curve known as the line spread function. A very sharp and narrow line spread function corresponds to a rapid edge response, ie, a rapid change in intensity. A more gradual change in pixel intensity will produce a broadened line spread function.

Figure 3 plots the edge responses for the two neck masses seen in Fig 1. Note that the gray scale intensity for the benign node changes rapidly as a function of position. In contrast, the malignant node visually has a poorly defined edge, and its edge response shows no distinct transition point.

The edge responses of neck masses will differ depending on their anatomic structure and their appearance on CT scan. Benign lymph nodes are relatively uniform in intensity and encapsulated. They should have a flat intensity profile within the node and a distinct border with surrounding tissue. The edge response for malignant nodes will be influenced by two factors. First, malignant nodes are characterized by a low-density center. This will produce a broad but definable edge response and moderate values for dispersion and variance. However, malignant nodes with extracapsular spread infiltrate adjacent tissue and have poorly defined borders. This should result in a broad or nondistinct edge response and very large dispersion and variance values.

Texture Analysis. Texture analysis attempts to quantify the patterns of gray scale distribution that compose an image.[10] Qualitative descriptors of texture that have physical correlates include terms such as coarse, fine, smooth, granular, and linear. Image texture is created by the distinct relationship between like and unlike pixels. A commonly used technique for texture analysis is based on a mathematic matrix known as the co-occurrence matrix. This matrix specifies the relationship between the gray scale intensity of adjacent pixels. For example, in this matrix, the element x_{ij} represents the number of occurrences in which a pixel of gray scale intensity i is adjacent to a pixel of gray scale intensity j in a given direction. A separate co-occurrence matrix is constructed for the pixel relationship in each of the 8 adjacent directions, and these matrices are averaged to produce the overall co-occurrence matrix. The co-occurrence matrix of an image in which adjacent pixels are similar will have large intensities clustered around the diagonal axis. On the other hand, an image of high contrast will possess many large transitions between gray scale intensities, and its co-occurrence matrix will therefore be dominated by off-axis values.

Haralick et al[11] described 14 texture features that can be calculated from the co-occurrence matrix to describe the spatial relationship of the gray scale values. For example, the angular second moment is the sum of the square of the entries in the co-occurrence matrix. A homogeneous image will have only a few gray scale transitions, and the angular second moment will be large. Another texture feature, known as the contrast, gives greater weight to off-axis values in the co-occurrence matrix and will therefore be larger for nonhomogeneous or "busy" images.

In their work, Haralick et al[11] used these variables to classify photomicrographs, aerial photographs, and satellite imagery. This technique has since been used by others for classification of medical images. For example, Morris[12] used texture analysis to compare ultrasound images of the human placenta. A co-occurrence matrix was constructed using a separation distance of 5 pixels. Significant differences were seen in certain texture parameters between smokers and nonsmokers and between normotensive and hypertensive women.

A modified texture method was used by Chandrasekaran et al[13] to analyze ultrasound images of cardiac amyloid and hypertrophic cardiomyopathy. The authors calculated gray level run length and gray level difference variables, based on variations in gray scale along the vertical and horizontal axes of the image. Variables calculated from the run length measurements, in particular the gray level nonuniformity, were significantly different for normal, hypertrophic, and amyloid hearts.

Texture analysis has also been used to examine nuclear chromatin granularity in human breast tissue, to look at Nissl substance in motoneurons, and to discriminate normal brain from edema and tumor in MRI scans.[14-16]

Fourier Analysis. Fourier transforms are commonly used to convert one-dimensional signals (eg, a sound wave) between the time domain and the frequency domain. Digital filtering, spectral analysis, and other calculations can be readily performed in the frequency domain. An image represents a two-dimensional signal in the spatial domain, and can therefore be converted to the frequency domain with a two-dimensional Fourier transform. The results of the transform will be a matrix of equal size to the original image. Each pixel in this matrix will have both a real and an imaginary component, sometimes also expressed in terms of amplitude and phase. Most representations of two-dimensional Fourier transforms display only the magnitude, although phase information must be preserved if an inverse Fourier transform is to be done. Typically, the frequency domain is displayed as an image with low-frequency components at the center of the image, and high spatial frequencies at the outer edges. The central pixel of a two-dimensional Fourier transform represents the DC component of the image and is usually several orders of magnitude greater than the surrounding elements.

Southard and Southard[17] used one-dimensional Fourier analysis to compare normal and osteoporotic rabbit humeri. They selected a linear array of pixels in the digitized radiographs and calculated the mean absolute value and mean first spatial moment of the Fourier transform of this array. Both parameters were significantly lower in osteoporotic bone, possibly due to changes in the trabecular coarseness. They also looked at variance in gray scale intensity along the same linear array, but did not see any significant differences between normal and abnormal bone.

If malignant masses are less uniform in appearance than benign masses, their images should contain more high spatial frequencies. According to this hypothesis, their Fourier transforms will therefore have a greater power spectrum in the high-frequency regions.

METHODS AND MATERIALS

Analysis was performed on 21 patients: 10 with head and neck SCCA and 11 with nonmalignant lymph nodes. All patients underwent a selective, radical, or modified radical neck dissection. Those patients with nodes seen on CT scan but subsequently found to be histologically clear of cancer make up the nonmalignant group. Preoperative CT scans of the neck were obtained on all patients. Scans were performed on a GE 9800 helical scanner following intravenous injection of an iodine-based contrast solution. Slice thickness was 3 mm at 3-mm intervals. The proprietary General Electric Medical Systems Image Extract Tool program was used to convert the raw data files into 512 × 512 image files with 12-bit intensity resolution.[18] Data files were then transferred via Ethernet to the various computers used for image analysis.

A common limitation in comparisons between radiographic and pathologic findings is ensuring that the neck mass analyzed on the CT scan is identical to the mass sectioned by the pathologist. Anatomic changes during resection and fixation, as well as the presence of multiple nodes in one region of the neck, can make this correspondence difficult. In this study, patients with multiple positive nodes in a single region were excluded. The analysis included only patients who had a single node seen on CT scan and a single positive node in the pathologic specimen in one clearly defined region of the neck. Analysis was therefore done on 1 mass for each patient. There were 11 nonmalignant masses and 10 malignant masses studied.

Much of the initial image display was performed with the public domain software NIH Image.[19] NIH Image was also used to select the regions of interest used for profile, histogram, and texture analysis. Since this program operates with 8-bit images, there was some loss of intensity resolution with these selections. For Fourier analysis, the 12-bit image data files were directly accessed and calculations were based on these higher-resolution pixel values.

A single representative scan that displayed a cross section of each patient's neck mass was selected. For histogram analysis, the best estimate of the border of the mass was manually outlined. NIH Image exported the pixel distribution of this region to a data file. A Microsoft Excel spreadsheet macro was used to calculate the histogram dispersion and variability.[20] Since precise borders can be difficult to identify, particularly if there is invasion of adjacent structures, this process was repeated 10 times to calculate a mean histogram dispersion and variance for each mass.

A custom algorithm was developed for edge analysis. The outline of the mass was first defined, as described above. The edge response along 16 line segments running radially outward from the center of the mass was then determined. The edge response was differentiated to obtain the line spread function. The point of maximum change (the peak of the line spread function) was determined. This point was constrained to lie within 10 pixels of the user-defined edge of the mass. The line spread function was then truncated to a length of 20 pixels, centered around the point of maximum change. Dispersion and variance were calculated for the line spread function. This process was repeated for each of the 16 segments around the circumference of the mass. The dispersion and variance values from these points were averaged for a mean dispersion and variance for each mass.

Texture analysis was done using the rectangular region of interest that best represented the central portion of each mass. The gray scale values from these regions were then extracted and texture features were calculated with the program Classify.[21] Calculations were made along directions pointing to each of the 8 nearest neighbors, and these values were then averaged. The pixel separation distance was varied from 1 to 9 pixels.

Regions of interest for Fourier transforms were selected in a similar fashion. All of the regions for Fourier analysis were, however, of equal height and width. In order to minimize zero-padding in the fast Fourier transform (FFT) algorithm, dimensions that were exponential powers of two were used as much as possible. The FFT and the magnitude of the transform were then calculated with the program Matlab.[22] The resulting matrix represents the power spectrum of the image.

The distribution of the power spectrum of the image was determined by calculating the relative ratio of the spectrum in the high spatial frequencies to the entire AC power spectrum. The total AC power spectrum was first calculated by summing all of the entries in the Fourier transform, excluding the DC component (the central pixel). The high-frequency components were then calculated by summing the magnitudes of progressively smaller quadrants along the outer regions of the transform matrix. The total magnitude in these outer quadrants was then divided by the total AC power to determine the overall contribution of high spatial frequencies to the image.

The width in the maximum dimension averaged 68.6 ± 23.9 pixels for the malignant masses and 25.2 ± 8.0 for the benign lesions. Each of the four analysis techniques used here is independent of the size of the region of interest.

RESULTS

Histogram Analysis. Results for histogram dispersion and variance for SCCA and benign nodes are shown in Table 1. The p values shown represent a comparison between the malignant and benign nodes, based on a two-tailed *t*-test. In contrast to the hy-

TABLE 1. RESULTS FOR HISTOGRAM ANALYSIS

Disorder	*Dispersion*	*p*	*Variance*	*p*
SCCA	0.76 ± 0.23		1.03 ± 0.55	
Benign node	0.88 ± 0.42	.42	1.55 ± 1.27	.25

SCCA — squamous cell carcinoma.

TABLE 2. RESULTS FOR EDGE RESPONSE ANALYSIS

Disorder	*Dispersion*	*p*	*Variance*	*p*
SCCA	2.32 ± 0.53		8.12 ± 2.78	
Benign node	1.32 ± 0.46	.0002	3.45 ± 2.29	.0004

pothesis, benign nodes actually had slightly higher values for gray scale dispersion and variance than SCCA, but the differences were not significant. Histogram analysis was therefore not useful in distinguishing these two groups of masses.

Edge Response Analysis. Both edge response dispersion and variance were significantly lower for benign nodes than for SCCA (Table 2). This result agreed with the hypothesis that malignant lesions form less distinct borders with adjacent tissue than benign nodes.

Texture Analysis. Thirteen different texture features were calculated by the Classify program for each of the neck masses at separation distances of 1 through 9 pixels. As the pixel separation distance was increased, changes were also seen in most of the texture features. However, these changes affected both malignant and benign masses in a similar manner. The greatest difference between the two groups was actually seen in most cases at a separation distance of only 1 pixel. Also, several masses were less than 15 pixels wide, so that texture features at large separation distances could only be calculated for the central portion of the mass. For these reasons, a separation distance of 1 pixel was used for comparison.

The complete results are shown in Table 3. There were differences at the p = .05 level, calculated with a two-tailed *t*-test, for 7 of the 13 features. Discriminant analysis was also performed on the 13 texture variables. Table 4 shows the pooled within-groups correlation matrix for these variables.

Because of the high correlation between many of the texture variables, a stepwise discriminant analysis using the F-value for the change in Wilks' lambda was performed. With an F-to-enter value of 3.84, only the Information Measure of Correlation 1 feature satisfied the criteria as a useful predictor variable. In other words, stepwise addition of the other variables did not significantly increase the ability to discriminate between the two groups of neck masses.

Fourier Analysis. The results of Fourier analysis are shown in Table 5. The Table entries represent the ratio of the power spectrum summed in the higher frequencies to the sum of the entire spectrum, excluding the central DC component. The high-frequency component was significantly larger for SCCA than for benign nodes, in agreement with the hypothesis that malignant node images have a greater percentage of high spatial frequencies.

DISCUSSION

Previous techniques for distinguishing malignant and benign neck masses have relied on basic image properties such as size, shape, and the general appearance of the mass. The identification of these features represents a simplified and qualitative form of image analysis. In this study, more sophisticated pattern recognition tools were used to analyze neck masses. Significant differences were seen between malignant and benign nodes in three of the four techniques tested.

Histogram analysis compared the basic distribution of gray scale density occurrence within an image. This distribution was not dependent on the shape or structure of the neck mass image or the spatial distribution of the pixels. Although early visual inspection of several malignant nodes led to the hypothesis that these images would show a greater distribution of gray scale values, this was not confirmed by the quantitative analysis. There was no significant difference in histogram dispersion or variance between benign and malignant nodes.

Edge analysis compared the gray scale intensity profiles present at the borders between the neck mass and its adjacent tissue. Comparison was based on the width of the line spread function. This function was obtained by differentiating the edge response,

TABLE 3. RESULTS FOR TEXTURE ANALYSIS

Texture Feature	*SCCA*	*Benign Node*	*p*
Angular Second Moment	0.18	0.12	.10
Contrast	0.51	1.63	.02
Correlation	0.88	0.81	.05
Variance	2.31	4.33	.06
Inverse Difference Moment	0.82	0.69	.01
Sum Average	9.66	8.43	.40
Sum Entropy	1.31	1.46	.1
Sum Variance	86.87	73.33	.6
Entropy	3.48	4.21	.05
Difference Entropy	1.08	1.55	.004
Difference Variance	0.84	1.42	.01
Information Measure of Correlation 1	−0.45	−0.35	.004
Information Measure of Correlation 2	0.92	0.89	.3

TABLE 4. POOLED WITHIN-GROUPS CORRELATION MATRIX FOR TEXTURE ANALYSIS VARIABLES

	ASM	*CON*	*COR*	*D ENT*	*D VAR*	*ENT*	*IDM*	*IMC 1*	*IMC 2*	*S AVG*	*S ENT*	*S VAR*	*VAR*
ASM	1.00												
CON	−0.60	1.00											
COR	0.15	−0.62	1.00										
D ENT	−0.77	0.93	−0.51	1.00									
D VAR	−0.71	0.92	−0.56	0.98	1.00								
ENT	−0.24	0.07	−0.05	0.13	0.11	1.00							
IDM	0.76	−0.96	0.52	−0.99	−0.95	−0.12	1.00						
IMC 1	−0.33	0.61	−0.84	0.67	0.72	0.13	−0.61	1.00					
IMC 2	−0.39	−0.11	0.76	−0.01	−0.11	0.05	−0.01	−0.69	1.00				
S AVG	−0.18	0.02	−0.04	0.07	0.05	1.00	−0.06	0.10	0.03	1.00			
S ENT	−0.93	0.63	0.03	0.77	0.69	0.18	−0.77	0.15	0.57	0.11	1.00		
S VAR	−0.32	0.31	0.00	0.26	0.23	−0.16	−0.30	−0.07	0.29	−0.18	0.46	1.00	
VAR	−0.67	0.71	0.07	0.77	0.71	0.05	−0.79	0.11	0.49	−0.01	0.84	0.33	1.00

ASM — Angular Second Moment, CON — Contrast, COR — Correlation, D ENT — Difference Entropy, D VAR — Difference Variance, ENT — Entropy, IDM — Inverse Difference Moment, IMC 1 — Information Measure of Correlation 1, IMC 2 — Information Measure of Correlation 2, S AVG — Sum Average, S ENT — Sum Entropy, S VAR — Sum Variance, VAR — Variance.

and was therefore very sensitive to small changes in intensity. In order to get a representation of the mass as a whole, 16 different edge responses around the circumference of the mass were calculated. Of the four image processing techniques studied here, edge analysis most closely duplicated techniques used visually to compare radiologic studies. Edge analysis also produced the greatest distinction between benign and malignant nodes. Results showed a significantly broader edge response for malignant masses than seen in benign neck masses. This agreed with the hypothesis that malignant masses often infiltrate surrounding tissue and subsequently have poorly defined borders. Benign nodes formed distinct borders with adjacent tissue in the neck and therefore had lower dispersion values.

The technique for texture analysis was based on the co-occurrence matrix, an algebraic description of the gray scale distribution between adjacent pixels. Texture features were calculated that described the numeric properties of this matrix. Of these 13 features, there were 7 that differed significantly between malignant and benign masses. The greatest difference between the two groups occurred in a variable called the Information Measure of Correlation 1, and a stepwise analysis showed that inclusion of this single variable was sufficient to discriminate between the benign and malignant masses.

TABLE 5. FOURIER TRANSFORM RESULTS

Disorder	*High Spatial Frequency Component**	*p*
SCCA	0.68 ± 0.99	
Benign node	0.41 ± 0.18	.0005

*Ratio of magnitude of summed magnitude of Fourier transform divided by total spectral magnitude, excluding central DC component.

The results of texture analysis are difficult to interpret, however, because unlike histogram or edge analysis, they do not correspond to an easily described visual pattern. Most of the features are strongly related to the predominance of on-axis components in the co-occurrence matrix. For example, the Difference Variance feature represents the variance in a linear array comprising the sums of the diagonals in the co-occurrence matrix. A uniform image produces a large on-axis co-occurrence matrix, which in turn results in a large Difference Variance. In this study, Difference Variance was seen to be significantly larger in benign nodes, in agreement with the overall model that benign nodes have greater uniformity. The Difference Entropy is the entropy of the same linear array. For these images the Difference Entropy was strongly correlated with the Difference Variance and was also significantly greater for the benign nodes.

Significant differences between benign and malignant nodes were also seen in the texture features Contrast and Inverse Difference Moment. These two features should be inversely related to each other, and the correlation matrix indeed showed the two to have a correlation of −0.96. However, the Contrast value was higher and the Inverse Difference Moment was lower for the benign mass, in disagreement with the assumption that benign masses have less variation in their gray scale values.

The greatest difference between benign and malignant masses was seen in the texture feature Information Measure of Correlation 1. This texture feature is the normalized difference between the Entropy of the entire co-occurrence matrix and the Entropy of the marginal probability matrices. The marginal probability matrices are linear horizontal and vertical arrays derived by summing the rows and columns, respectively, of the co-occurrence matrix. This texture feature should be greater for images with large contrast changes. For these images, the Information Measure of Correlation 1 was greater for the malignant masses, in agreement with the hypothesis that benign nodes have a more uniform texture appearance.

In summary, differences in texture features for benign and malignant nodes, when present, appeared to occur in an arbitrary fashion. Although the differences were in some cases significant by statistical criteria, the texture values themselves did not follow a consistent pattern. In some cases, features typical for noisy images were greater for benign nodes; in other cases, similar features were greater for the malignant nodes. It is therefore difficult to recommend the use of texture analysis for discrimination of the neck mass images.

Fourier analysis is an especially powerful method for discerning repetitive patterns within a signal. However, it is also useful in determining the spectral distribution of gray scale intensity within a nonrepetitive image. The Fourier transforms of all of the neck masses showed a rapid drop in power as a function of frequency, indicating that most of the image was contained within the low spatial frequencies. The malignant nodes were found to have a significantly greater fraction of their signal in the higher spatial frequencies than the benign nodes. This agreed with the hypothesis that the inhomogeneities often seen in images of malignant masses would result in higher spatial frequencies.

CONCLUSION

Statistically significant differences were seen between benign and malignant nodes on edge analysis and Fourier analysis. Differences were also seen on texture analysis, although these results were less consistent and therefore more difficult to interpret. It is concluded that automated image processing techniques such as the ones studied here can be additional tools for the clinician to use in extracting information from medical images and diagnosing neck masses.

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PROGNOSTIC FACTORS IN SUPRAGLOTTIC CARCINOMA PATIENTS TREATED BY SURGERY OR RADIOTHERAPY

CARLOS R. SANTOS, MD
LUIZ P. KOWALSKI, MD, PHD
JOSÉ MAGRIN, MD
RICARDO C. FOGAROLI, MD
JOÃO C. R. SANTOS, MD
MURILO V. SABÓIA, MD
HUMBERTO TORLONI, MD
SÃO PAULO, BRAZIL

The decision regarding treatment of supraglottic carcinoma remains controversial, despite recent advances in radiotherapy and surgical procedures. The need to evaluate the prognostic importance of demographic, clinical, pathologic, and treatment-related variables prompted this study. The patient population was of 164 consecutive cases of supraglottic carcinoma. Clinical charts were reviewed and cases were restaged according to the 1987 version of the UICC-AJC classification based on the initial clinical description: 18 T1, 30 T2, 58 T3, 58 T4, 61 N0, 24 N1, and 79 N2a-N3. Management policy for these patients varied during the study, reflecting different opinions of the responsible physicians and technical advances. Seventy-seven patients (47%) underwent surgery, whereas the remaining 87 (53%) were submitted to radiotherapy as the first treatment. At the study closing date, 47 and 4 of the patients who underwent surgery and radiotherapy, respectively, remained alive with no evidence of disease. The 5-year actuarial survival rates were 58.5% for patients initially treated by surgery, and 16.3% for patients in the radiotherapy group. Although several demographic, clinical, and pathologic variables were studied, only initial treatment ($p < .0001$), N stage ($p = .0003$), and T stage ($p = .0017$) were deemed to have independent prognostic value by multivariate regression techniques based on Cox's proportional hazards model. This study has shown that survival of supraglottic carcinoma patients depends on the treatment modality, T stage, and N stage. Our results are disappointing with regard to radiotherapy as a treatment for supraglottic carcinoma in patients with tumors at clinical stages III and IV.

KEY WORDS — larynx carcinoma, supraglottis, survival analysis, treatment.

INTRODUCTION

The decision regarding treatment of supraglottic carcinoma remains controversial. Surgery and radiotherapy, alone or in combination, are the mainstays of treatment of these tumors.[1-11] Selection of the treatment relies on certain factors: tumor site, clinical stage, the patient's overall medical condition, the philosophy and experience of the physician, and the patient's choice.

Supraglottic squamous cell carcinoma (SCC) follows a typical pattern characterized by local invasion, extension to the hypopharynx or vallecula, and substantial changes in the prognosis due to the higher proportion of lymph node metastases when compared to tumors confined to the glottis.[2,8,10-13] Despite the severity of the clinical presentation, it is a potentially curable disease, especially when diagnosed at early stages.[1,3-5,12,13] Analysis of survival rates is the most important index of therapeutic efficacy. Although stage has long been accepted as the best single determinant of survival for supraglottic carcinoma, the prognosis is complex and may depend on several other risk factors.[1,7,10,11]

The need to evaluate the prognostic importance of demographic, clinical, pathologic, and treatment-related variables in a series of 164 patients with supraglottic carcinoma treated at a single institution prompted this study. In the future, the significant factors that influence disease control, identified by multivariate survival analysis, will be used to improve the selection criteria of patients for the different treatment options.

PATIENTS AND METHODS

We reviewed the records of 164 consecutive patients with recently diagnosed SCC arising in the supraglottis, referred to the head and neck surgery department of the Hospital A. C. Camargo, São Paulo, Brazil, from 1965 to 1990. All diagnoses were confirmed before treatment started. Patients' ages ranged from 33 to 81 years (median 56 years). There were 147 men (90%) and 17 women (10%). The areas involved by the primary tumor included the epiglottis (99 cases), false vocal cords (100 cases), ventricle (89 cases), and arytenoid (88 cases). Tumor extension to the glottis was found in 45 cases, to the pyriform sinus in 49 cases, and to the base of the tongue in 42 cases. The vocal cord mobility was reduced in 30 cases and the hemilarynx was fixed in

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From the Departments of Head and Neck Surgery (C. R. Santos, Kowalski, Magrin, J. C. R. Santos, Sabóia), Radiotherapy (Fogaroli), and Research (Torloni), Hospital A. C. Camargo, Fundação Antonio Prudente, São Paulo, Brazil.

CORRESPONDENCE — Luiz P. Kowalski, MD, PhD, Dept of Head and Neck Surgery, Hospital A. C. Camargo, Fundação Antonio Prudente, R. Prof. Antonio Prudente, 211, 01509-010 São Paulo, Brazil.

TABLE 1. SITES OF RECURRENCE AND SALVAGE TREATMENT ACCORDING TO TREATMENT GROUP

		Surgery Group		*Radiotherapy Group*	
Categories		*No.*	*%*	*No.*	*%*
Recurrences	None	51/77	66	16/65	25
	Local	7/77	9	34/65	52
	Neck	11/77	14	27/65	42
	Distant	12/77	16	7/65	11
Salvage	None	14/26	54	28/49	57
	Laryngectomy (±ND)	0/26	0	5/49	10
	ND	2/26	8	5/49	10
	Radiotherapy	9/26	35	2/49	4
	Chemotherapy	1/26	4	9/49	18

ND — neck dissection.

54. Patient charts were reviewed, and the cases were restaged with the 1987 version of the UICC[14] classification based on the initial clinical description: 18 T1, 30 T2, 58 T3, 58 T4, 61 N0, 24 N1, and 79 N2a-N3. At the time of diagnosis, none of the patients presented distant metastasis.

The management policy for these patients varied during the study, reflecting different opinions and skills of the responsible physicians and some technical advances. Seventy-seven patients (47%) underwent surgery, whereas the remaining 87 (53%) were submitted to radiotherapy as the first treatment. Initial surgical treatment included total laryngectomy (TL) in 30 patients, TL and partial pharyngectomy in 30, supraglottic laryngectomy in 15, and epiglottectomy in 2. A total of 69 patients underwent neck dissection (26 bilateral) concomitantly with primary tumor resection. Postoperative radiotherapy was used as an adjuvant to surgery in 17 cases (in 1 because of involved surgical margins and in 16 due to positive lymph nodes). Postoperative radiotherapy consisted of a wide field technique encompassing both sides of the neck, with a dose range from 50 to 65 Gy.

External beam radiation was administered by means of cobalt 60 (20 cases) or a 4-MeV linear accelerator (67 cases). Patients were treated with two parallel opposing wide portals with a source-to-skin distance of 80 cm and with the dose calculated at midline. Regional lymph nodes on both sides were always included in the portals. A boost to the neck was used in patients with clinically positive nodes. A total dose of 12 to 86 Gy (median 70 Gy) was given to the target volume in daily fractions of 2 Gy, 5 times a week. Twenty-two patients were excluded from further analysis, because they had received a total dose of less than 60 Gy.

Statistical Analysis. Patients were divided into two groups according to initial treatment (surgery or radiotherapy). Product-limit estimates of the survivorship function were used for computation of the cumulative survival rates.[15] The overall survival (OS) time was defined as the interval between the date of surgery or the beginning of irradiation and the date of the last consultation (for censored observations) or date of death (for uncensored observations). Cox's regression techniques were used to build models representing the most parsimonious subset of variables with independent predictive properties with respect to the risk of death.[16] Inference was based on the partial likelihood ratio statistic between nested models (10% significance level).

RESULTS

The survival analysis includes only the 142 patients who completed the initially planned treatment. The 22 patients who received less than 6,000 cGy and were excluded from analysis died before 5 years. At the study closing date, there had been 6,211 patient-months of cumulative follow-up experience (mean 44 months). Seventy-five patients (53%) died of cancer recurrence or treatment complications, and 10 (7%) died of causes that were not cancer-related. At the study closing date, there were 51 patients (36%) alive with recent follow-up information (1 with recurrent cancer). An additional 6 patients (4%) were considered lost to follow-up.

Seventy-five patients presented 98 tumor recurrences (41 local, 38 neck, 19 distant; Table 1). Surgery was more effective than radiotherapy to control disease at the primary site (91% versus 48% local control rate). There were 27 cases (42%) with residual or recurrent neck disease among the patients who underwent radiotherapy. Of the 77 cases submitted to surgical treatment, 11 (14%) presented neck recurrence (5 on the dissected side of the neck, and 6 on the undissected side). Distant metastases were only found in 19 patients and were associated with tumor recurrence above the clavicles in 10 cases. In 17 patients (12%), a second primary tumor was diagnosed: oral cavity (3 cases), tonsil (4 cases), esophagus (2 cases), stomach (2 cases), lung (4 cases), skin (1 case), or urinary bladder (1 case).

Tumor control was achieved through initial surgery or irradiation in, respectively, 66% and 25%. Surgical salvage was attempted in 12 patients: TL and neck dissection (2 cases), TL (3 cases), and neck dissection (7 cases). Eleven patients underwent irradiation or chemotherapy and irradiation, and 42 were not considered candidates for further treatment because of the advanced stage of recurrence (Table 1).

Table 2 shows a summary of survival data for se-

TABLE 2. SURVIVAL RATES ACCORDING TO SELECTED DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

Variables	*Categories (Surgery/RT)*	*Surgery 5-y OS*	*p*	*RT 5-y OS*	*p*
Sex	Male (73/57)	56	.1789	19	.4047
	Female (4/8)	100		18	
Age	<50 (21/14)	66	.5362	0	.2985
	50-69 (51/44)	54		20	
	≥70 (5/7)	75		51	
T stage	T1 (7/10)	100	.1741	18	.0016
	T2 (14/12)	70		71	
	T3 (30/17)	62		0	
	T4 (26/26)	41		11	
N stage	N0 (37/20)	78	.0060	41	.0128
	N1 (14/9)	13		15	
	N2a-N2b (16/10)	57		15	
	N2c (8/14)	45		11	
	N3 (2/12)	50		8	
Clinical stage	I (3/3)	100	.3032	100	.0150
	II (6/6)	83		80	
	III (25/10)	66		12	
	IV (43/46)	49		14	
Sites involved					
Epiglottis	No (32/23)	60	.9291	44	.0388
	Yes (45/42)	58		10	
False vocal cords	No (26/26)	73	.1493	36	.1484
	Yes (51/39)	52		13	
Arytenoid	No (35/35)	84	.0099	32	.1545
	Yes (42/30)	40		6	
Vocal cord	No (53/47)	56	.6757	26	.0462
	Yes (23/18)	62		8	
	Ignored (1/0)	100		0	
Pyriform sinus	No (54/48)	69	.0072	2	.0262
	Yes (23/17)	34		18	
Base of tongue	No (62/49)	64	.1360	27	.1327
	Yes (15/16)	39		7	
Vocal cord mobility	Normal (33/29)	67	.1734	37	.0024
	Impaired (11/15)	50		0	
	Fixed (29/17)	58		20	
	Ignored (4/4)	0		0	

RT — radiotherapy, OS — overall survival.

lected demographic and clinical characteristics stratified by treatment group. No differences in survival rates according to sex or age were found. Although there was a tendency toward poorer prognosis as tumor stage increased from T1 to T4 in the group of patients treated by surgery, these differences failed to reach statistical significance. Patients with clinically positive metastatic lymph nodes exhibited poorer OS than those with no evident metastatic spread to cervical lymph nodes. Survival rates for clinical stage I and II patients, treated either by surgery or radiotherapy, were 100% and over 80%, respectively. The survival rates of stage III and IV patients who underwent radiotherapy were extremely low (12% to 14%) when compared to those of stage III and IV patients submitted to surgery as the first treatment (49% to 66%). Patients with tumor involving the pyriform sinus tended to have a worse prognosis than those in whom this site was not involved. The involvement of the arytenoid significantly reduced the OS of the patients treated by surgery. Patients with vocal cord fixation submitted to radiotherapy had a worse prognosis than those with no fixation. None of the other studied sites of involvement had appreciable effects on prognosis.

Table 3 shows 5-year survival rates according to pathologic variables. There was a tendency toward poorer prognosis in patients treated by surgery, because of carcinomas classified as grade II or III and

TABLE 3. SURVIVAL RATES ACCORDING TO SELECTED PATHOLOGIC CHARACTERISTICS

Variables	*Categories (Surgery/RT)*	*Surgery 5-y OS*	*p*	*RT 5-y OS*	*p*
Histologic grade	I (38/14)	61	<.0001	13	.1016
	II-III (17/3)	21		50	
	Not specified (20/46)	85		24	
	Undifferentiated (2/2)	0		0	
Surgical margins	Uninvolved (58/NA)	52	.8820	NA	NA
	Involved (1/NA)	100			
	Ignored (18/NA)	56			
Positive neck nodes*	pN0 (28/NA)	80	.0009	NA	NA
	pN+ (41/NA)	38			

NA — not applicable.

*Eight patients were not submitted to neck dissection as part of initial surgical treatment.

metastatic nodes. The prognostic effect of surgical margins in the surgical group and of tumor grade in the radiotherapy group of patients cannot be fully estimated, due to absence of data.

Survival rates varied significantly according to the initial treatment. Patients admitted to surgery had higher survival rates than those initially treated by radiotherapy. The radiotherapy equipment had a significant prognostic effect. Patients treated with cobalt equipment had higher survival rates than patients treated with a linear accelerator (Table 4).

In a subsequent step of the analysis, multivariate predictive models were built with Cox's regression technique (Table 5). Of the several candidate prognostic factors, only the presence of metastatic lymph nodes and involvement of the arytenoid were selected as independent predictors of the risk of death among patients submitted to surgery. Results of the radiotherapy group of patients were affected by T stage, involvement of the epiglottis, tumor extension to the vocal cords, and type of equipment.

DISCUSSION

The decision regarding proper management of primary supraglottic carcinoma remains controversial despite the most recent advances in radiotherapy and surgical procedures. For management, several approaches are used: surgery followed by radiotherapy in selected cases, preoperative irradiation and surgery, radiotherapy with salvage surgery for failures, and induction chemotherapy and surgery or irradiation according to tumor response.[1-13,17-19] The major difficulty for comparison of the reported results of the various treatment modalities are differences due to several other factors: age, sex, site, stage, histology, and lymph node metastasis. Most of the reported prognostic factors are interrelated, and their independent predictive effects cannot be fully demonstrated by crude univariate or stratified analy-

TABLE 4. SURVIVAL RATES ACCORDING TO SELECTED TREATMENT CHARACTERISTICS

Variables	*Categories (Surgery/RT)*	*Surgery 5-y OS*	*p*	*RT 5-y OS*	*p*
Decade of admission	1960s (13/7)	62	.8291	64	.1236
	1970s (21/29)	66		17	
	1980s (43/29)	53		18	
Initial treatment	(77/65)	59		21	
Neck dissection	No (8/NA)	83	.8032	NA	NA
	LND (1/NA)	100			
	Bilateral LND (6/NA)	60			
	RND (42/NA)	55			
	Bilateral RND (17/NA)	53			
	RND + LND (3/NA)	67			
Postoperative radiotherapy	No (60/NA)	51	.6842	NA	NA
	Yes (17/NA)	61			
Radiation doses	60-69 Gy (NA/18)	NA	NA	38	.1468
	≥70 Gy (NA/47)			15	
Radiotherapy equipment	Cobalt (NA/13)	NA	NA	48	.0368
	LA (NA/52)			15	

NA — not applicable, LND — lateral neck dissection, RND — radical neck dissection, LA — linear accelerator.

TABLE 5. COX REGRESSION MODELS FOR RISK OF DEATH

Variables	*Categories*	*Odds Ratio*	*p*
Model for patients initially treated by surgery			
Positive neck nodes	pN0	1.0	.0011
	pN+	3.8	
Arytenoid	Uninvolved	1.0	.0044
	Involved	2.6	
Model for patients initially treated by radiotherapy			
T stage	T1	1.0	.0004
	T2	0.2	
	T3	1.5	
	T4	1.5	
Epiglottis	Uninvolved	1.0	.0214
	Involved	2.4	
Equipment	Cobalt	1.0	.0486
	Linear accelerator	2.4	
Vocal cord	Uninvolved	1.0	.0508
	Involved	1.9	

sis.[7,10,12]

In our institution, since 1970, surgery has been the first choice as initial treatment for supraglottic carcinoma, with radiotherapy indicated during the postoperative period of selected cases (involved surgical margins and metastatic neck lymph nodes). Radiotherapy as initial treatment has been indicated for patients who are poor surgical risks, have unresectable tumors (in the majority due to fixed lymph node metastasis), or refuse surgical treatment. Consequently, patient selection has significantly impaired the results of radiotherapy. Although comparisons of OS are difficult to assess because of these differences in indications, variations in the rates of local and regional control can be very significant. The significant factors that influence disease control can be used to improve the criteria for treatment planning.

In most patients included in this series, cancer recurrence was the cause of death. The larynx itself and the neck were the most frequent sites of failure. Patients submitted to irradiation had a significantly higher number of local and neck recurrences than those initially treated by surgery. Local recurrences after surgical treatment result mainly from inadequate margins of resection. The main reasons for irradiation failure are unrecognized local tumor extensions and substandard doses. It is noteworthy that of the patients submitted to surgery and irradiation who presented recurrences, 54% and 57%, respectively, were not considered for further treatment because of the advanced stage of local, regional, and/or distant disease. The main reasons for the delay of diagnosis of recurrence after radiotherapy can be attributed to poor patient compliance as to follow-up visits, and difficulties in the management of patients with persistent edema. In most cases, local recurrences were not identified until there was some degree of airway obstruction. A policy of close follow-up for all irradiated patients and the decision to submit those with persistent edema, or even minor mucosal abnormalities, to direct laryngoscopy and biopsy under general anesthesia is mandatory.

Of the patients included in this series, 63% presented clinically positive lymph node metastasis. Neck recurrences were far more common in the radiation-treated group than in the surgical group. This suggests that surgery is a better choice for local and regional disease control. Considering that clinical evaluation is not accurate, there is a risk of unselectively waiting for clinically negative nodes to become positive. The elective treatment of the neck using at least a lateral neck dissection (levels 2, 3, and 4) for staging is considered essential in the therapeutic plan of all supraglottic cancers. A comprehensive neck dissection, either radical or modified, is advisable for all patients with clinically metastatic lymph nodes, because there is a risk of involvement of lymph nodes other than those at levels 2, 3, and 4. A bilateral neck dissection is required whenever the tumor involves the epiglottis or the preepiglottic space, due to the high incidence of contralateral metastases.[6,11,13]

Among the candidate prognostic factors studied in the group of patients submitted to surgical treatment, two constituted the most important determinants of risk of death on the basis of both the strength and the magnitude of the associations with the OS: involvement of the arytenoid and presence of metastatic lymph nodes. The risk of death for the radiotherapy group was affected by T stage, involvement of the epiglottis and vocal cords, and type of equipment.

In the present series, the number of positive nodes was an independent prognostic indicator. Patients with positive nodes were under higher risk of death than those with no lymph node involvement. The implication of this characteristic for prognosis has been previously documented.[10,11,20] Patients with clinically positive nodes should be submitted to a comprehensive neck dissection, independent of nodal site and size, because there is a considerable risk of involvement of multiple nodes and of extracapsular spreading. Both characteristics can only be ascertained by histologic examination. Extracapsular spreading was not analyzed in this study, because it has been routinely described in pathologic reports since the early 1980s.

The risk of death in the radiotherapy group of patients varied according to the sites of involvement and N stage. Similar results have been reported by others.[1,4] The better results obtained with cobalt 60 as opposed to a linear accelerator cannot be attributed only to the equipment. The significant worsening of the prognosis perceived during the last 20 years also cannot be attributed to irradiation, because it did not change significantly over this period of time in our institution. Patient selection was the best explanation for this finding, because 22 patients initially treated by radiotherapy were not able to complete the planned treatment, and some of them died during treatment as a result of the far-advanced stage of disease. Further, most patients with N2c and N3 stages underwent radiotherapy.

This study has confirmed that the use of information other than disease stage indices may constitute a major asset for our ability to predict survival of patients with supraglottic carcinoma. Since some of the prognostic factors represent clinical characteristics and tumor-related conditions that can be disclosed at diagnosis, they may be used to estimate expectancy of risk levels for subsequent optimization of treatment. Surgery is the optimal option for stage III or IV SCC of the supraglottis, and a special consideration to neck dissection cannot be overemphasized. Either primary radiotherapy or surgery is indicated in tumors at stage I or II. It seems reasonable to indicate radiotherapy for early tumors in patients considered suitable for a supraglottic laryngectomy but with medical contraindications to surgery. Surgery remains mandatory whenever there are positive lymph nodes in the neck.

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LARYNGOMUCOCELE AS AN UNUSUAL LATE COMPLICATION OF SUBTOTAL LARYNGECTOMY

CASE REPORT

XAVIER CARRAT, MD
JEAN-MARC FRANÇOIS, MD
FRANÇOIS DEVARS, MD
DOMINIQUE CARLES, MD
LOUIS TRAISSAC, MD
BORDEAUX, FRANCE

We report an unusual case of laryngomucocele occurring after subtotal laryngectomy. Laryngoceles generally have a congenital origin in a long-preexisting saccule, and their association with laryngeal carcinoma is well known. Laryngocele is usually favored by the increase of intraglottic pressure caused by the laryngeal carcinoma. However, an iatrogenic secondary laryngomucocele occurring after a surgical procedure is uncommon. We report in detail the physiopathologic conditions leading to the creation of this lesion.

KEY WORDS — laryngeal carcinoma, laryngomucocele, subtotal laryngectomy.

INTRODUCTION

The dilatation of the laryngeal ventricle, for which the term "laryngocele" was first introduced by Virchow in 1867, appears to be a very rare condition. Its occurrence has been estimated at approximately 1 per 2,500,000 population per year in the United Kingdom.[1] Usually, it consists of a dilatation of the sacculus of the laryngeal ventricle — an upward extension to the laryngeal ventricle arising from the roof of the ventricle between the false vocal cord and the inner surface of the thyroid ala. The normal saccule can extend as high as the superior border of the thyroid cartilage. It is now well known that laryngoceles may be internal, external, or combined. An internal laryngocele is confined to the interior of the larynx, whereas an external laryngocele protrudes through the thyrohyoid membrane up to the neck. A combined laryngocele is defined as an external laryngocele in association with a dilatation of the internal component. They may be unilateral or bilateral, and they may become infected, forming a laryngopyocele. Holinger and Brown[2] differentiate a laryngocele from a saccular cyst on the basis of a communication of the former with the laryngeal lumen and contents. The saccular cyst is filled with mucus, whereas the laryngocele is an air-dilated saccule. Saccular cysts are more commonly present in infants and children.[3] Occasionally, a laryngocele may be distended with mucus produced by the mucous gland near the aperture of the saccule, making it a laryngomucocele.

A large number of primary laryngoceles, with or without a laryngeal anomaly, have been reported. A laryngomucocele in association with laryngeal cancer seems to be a very rare condition, and only a few cases have been reported.[4,5] All of these cases were of primary origin. A description of a saccular mucocele occurring after treatment for laryngeal cancer was reported in 1973 by Cassady and Mathur,[6] but was related to local recurrence of the laryngeal neoplasm. To our knowledge, it is the first case report of this unusual complication after subtotal laryngectomy. The purpose of this report is to present the case of an iatrogenic secondary laryngomucocele occurring after a surgical procedure and to discuss the physiopathological conditions leading to the creation of this lesion.

CASE REPORT

A 68-year-old woman was admitted to the otolaryngology department in September 1990 for treatment of a T1bN0M0 glottic carcinoma. She had a history of chronic dysphonia for about 8 months, without any tobacco or alcohol history. She underwent a laryngoscopy with laryngeal videoendoscopy showing a well-differentiated squamous cell glottic carcinoma extending from the entire right true vocal cord to the anterior opposite cord. The right ventricle of Morgagni was also invaded. No computed

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From the Departments of Otolaryngology–Head and Neck Surgery (Carrat, François, Devars, Traissac) and Pathology (Carles), Pellegrin Hospital, University Bordeaux II, Bordeaux, France.

CORRESPONDENCE — Xavier Carrat, MD, Clinique Universitaire ORL 11A2, Hôpital Pellegrin, Place Amélie Raba Léon, 33076 Bordeaux, France.

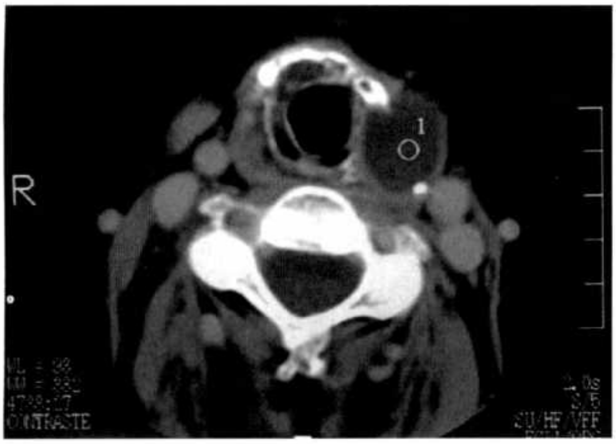

Fig 1. Axial contrast computed tomography scan at cricohyoidopexy level. On left, laryngomucocele extends to adjacent cervical area.

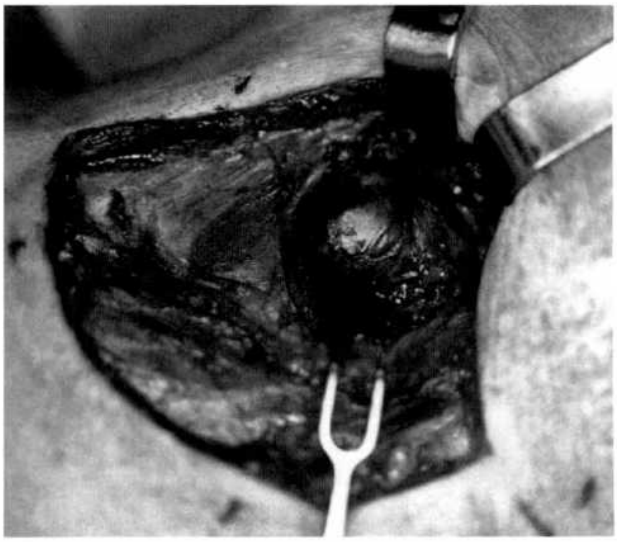

Fig 2. Intraoperative photograph showing laryngomucocele.

tomography (CT) scan evaluation was made, and she underwent only laryngeal tomograms. A surgical procedure was decided on, and a cricohyoidoepiglottopexy as described by Piquet et al[7,8] and Majer and Rieder[9] was performed. She was well until February 1996, when a left cervical mass 3 × 2 cm in size was discovered behind the hyoid bone and below the submaxillary gland. This cervical tumor, exactly located in the previous surgical pexy, was painless but tender, smooth, and fixed to the underlying tissue. A fiberscopic evaluation of the neolarynx was not contributive. She underwent CT revealing a heterogeneous spheric mass with a peripheral ring, lateral to the neolarynx, without any involvement of its lumen or mucosa (Fig 1). No endolaryngeal recurrence was shown during a new laryngoscopy with histologic analysis. A laryngocele was then suspected, and a left cervicotomy was performed. There was a large laryngomucocele positioned between the hyoid bone and the upper border of the cricoid ring (Fig 2). The mucocele contained a brownish gray, inspissated mucus identical to the fluid found in a sinus mucocele. A complete removal was performed without effraction of the laryngeal mucosa. The patient had an uneventful recovery without any aspiration or phonation disorders.

Microscopic examination of the surgical specimen confirmed the diagnosis of laryngocele and showed that the mucocele was lined by a pseudostratified ciliated columnar respiratory epithelium with mucous glands but without any carcinomatous recurrence or metaplasia (Fig 3).

DISCUSSION

Most laryngoceles are probably of congenital origin, but they may be induced by a pronounced increase in intralaryngeal pressure, occurring, for ex-

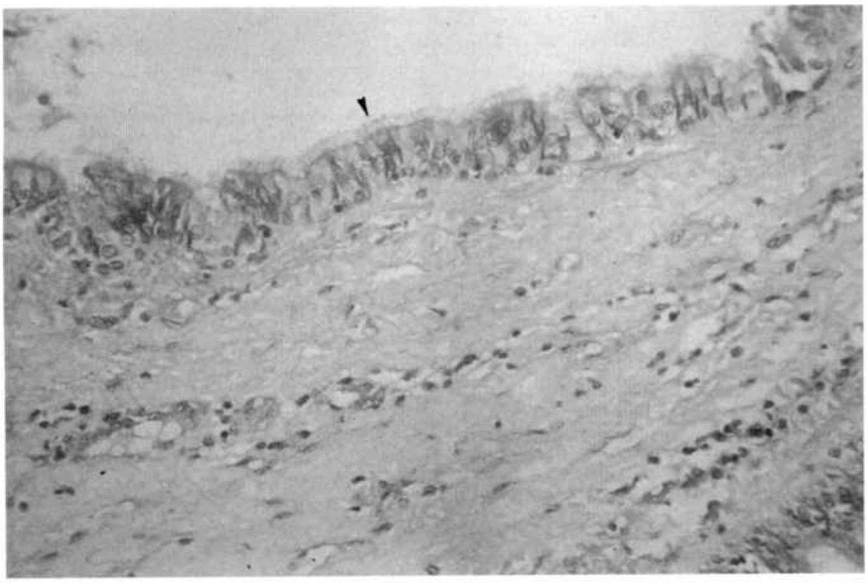

Fig 3. Histologic examination of laryngocele with ciliated epithelium (arrowhead) and underlying mucous glands (H & E, original ×160).

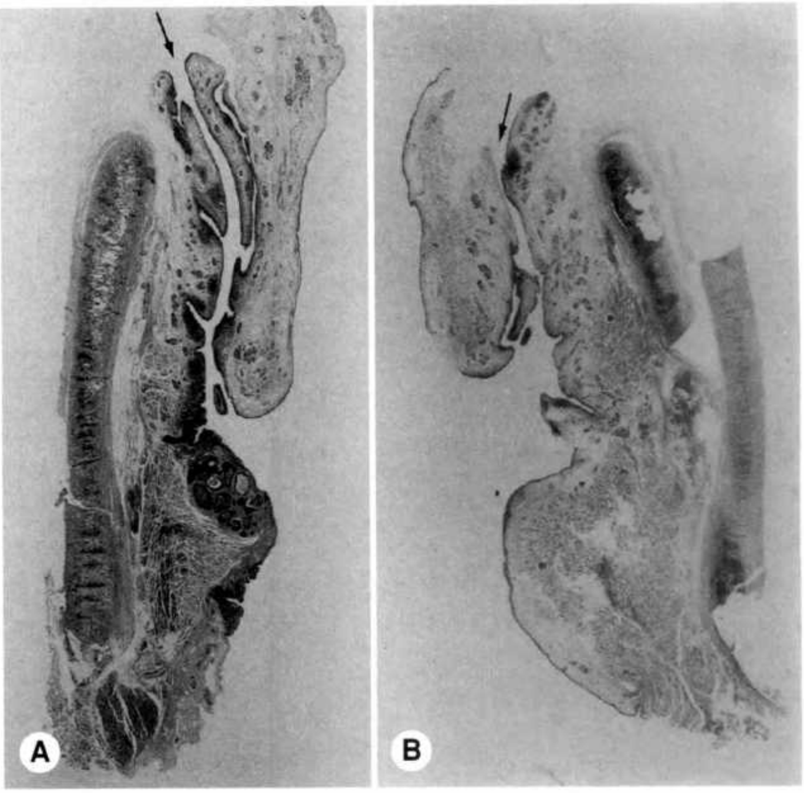

Fig 4. Serial coronal sections of larynx. Arrows — transected head of laryngocele. A) On left, vocal cord carcinoma with transected laryngocele connected to ventricle via nondilated segment of intralaryngeal segment. B) On right, external laryngocele extending up superior border of thyroid cartilage without evidence of carcinoma.

ample, with glass blowing or wind instrument playing. The first description was in a muezzin and reported by Larrey, a surgeon in Napoleon's army during the Egyptian campaign. Inflammation and laryngeal tumors seem also to play a role, probably producing a flap-valve mechanism, but their association remains controversial.

Undoubtedly, the incidence of laryngocele associated with laryngeal cancer is higher than that of asymptomatic laryngocele demonstrated only with CT. Many authors have reported cases of laryngeal cancer associated with laryngocele and have suggested that cancer may play a part in the development of ventricular diverticuli. Micheau et al[10] reported a 2% incidence of laryngocele in larynges removed for pharyngeal cancer, and 18% in larynges removed for laryngeal cancer, for an overall incidence of 12%. In comparison, Close et al[11] found an incidence of laryngocele of 12.5% in 304 consecutive patients studied by CT of the larynx without clinical suspicion of laryngocele. It was asymptomatic and present in 8.6% of asymptomatic patients undergoing CT for miscellaneous reasons, whereas 28.8% of patients with a laryngeal cancer have laryngocele. Sometimes, however, cancer grows on a preexisting or simultaneously developing laryngocele, making it of particularly serious gravity because of the deep laryngeal invasion in the paraglottic space. Tumor arises within the laryngocele and spreads early to the preepiglottic space and laryngeal surface of the epiglottis, and then to the paraglottic space when the ventricular mucosa is involved.

All these previously reported results suggest that the incidence of laryngoceles is higher in association with laryngeal cancer and that it may play a role in their development. The coexistence of both suggests a direct relationship, the laryngocele resulting from an increased intralaryngeal pressure secondary to the presence of cancer and the modification of airway patency. An individual and predisposing anatomic variation in the size of the saccule contributes to an occasional expansion of this structure when the intralaryngeal pressure increases. Broyles[12] found at autopsy of 50 adult larynges that 7% of saccules in normal larynges were 15 mm long or even longer. Gerard-Marchand et al[13] have investigated the height of the ventricle in two groups of laryngectomies: the first for laryngeal carcinoma and the second for hypopharyngeal tumor. They found that the ventricles in patients of the first group were deeper than those of the second group, with a statistically significant difference. They attribute this difference to the increased laryngeal pressure consequent upon tumoral glottic obstruction. This work was confirmed later by Birt.[14] In our case report, the absence of any risk factor and the history of chronic dysphonia suggest a chronic laryngitis that has degenerated. Increased laryngeal pressure secondary to the dysphonia and vocal strain probably favored the growth of a preexisting dilated and bilateral saccule. In the same way, preexisting dilated saccules were bilateral in our case, even though the carcinomatous lesion was only on the left vocal fold, suggesting an acquired glottic carcinoma and preexisting laryngoceles. The previously reported incidence of ipsilateral relationship between tumor and laryngocele varies between 47% and 57%, indicating that laryngoceles are not always dependent on the tumoral growth.[10,11,15,16] Other factors, such as hoarseness, cough, chronic inflammation due to dysphonia or breathing and pressure disorders, are responsible for the genesis of these lesions and may or may not be related to the presence of tumors with associated and predisposing anatomic factors.

Primary laryngomucocele in conjunction with cancer seems to be a very rare condition, and in all these reports the mucocele and the cancer were concomitant. The first case was reported by Harrison[5] in 1977. More recently, a bilateral primary laryngomucocele in association with laryngeal cancer was described by Gregor et al.[4] They stated that the obstruction of the saccule's mouth by a spreading tumor results in an accumulation of mucus and induces these saccular mucoceles. The conditions for the development of an iatrogenic laryngomucocele after a subtotal laryngectomy are identical. During the surgical procedure, we transected a deep preexisting saccule or external laryngocele extending beyond the upper border of the thyroid cartilage and close to the hyoid bone. An examination of the serial sections of both sides of the larynx confirmed an external laryngocele, extending up to the superior border of the thyroid cartilage, with the head lacking (Fig 4). The head of the preexisting saccule was not removed and enclosed in the cricohyoidopexy. A CT scan showed — on the opposite side, beside the left laryngomucocele — the remaining right saccule behind the hyoid bone. The fibrous scar had blocked the mouth of the remaining saccule head. Then, like a congenital cyst, its pseudostratified and ciliated epithelium, invested with closely set mucous glands, induced an accumulation of contained mucus. The accumulation of firm mucus in a closed cavity explains this tender cervical mass and its development in the zone of lower external resistance. External removal by cervicotomy was in this case the only feasible procedure, because of the medial bony wall created by the cricohyoidoepiglottopexy.

This complication remains unusual but not exceptional. In our experience, it is the only case in a partial laryngectomy, with an incidence of 2%. Yet, 2 other cases were reported by Laccourreye et al[17] in 1994 after a modified but similar surgical procedure (tracheocricohyoidoepiglottopexy) with a probably similar developmental spectrum. Other partial laryngectomies may also be implicated. A laryngocele occurred and was diagnosed in our institution 10 months after a horizontal glottectomy like the one described by Calearo and Teatini and was excised by endoscopic laser.[18] A third one was diagnosed on the same side 1 year after a frontolateral thyrotomy for a left glottic carcinoma with ablation of the anterior part of the false vocal cord. In these 2 cases, a concomitant excision of the mouth of the saccule, in association with the ventricle and the anterior part of the false vocal cord, produced a scarring process of the saccule orifice, resulting in a flap-valve mechanism and an iatrogenic and delayed dilated saccule. Review of previous laryngeal sections failed to discover any preexisting dilated saccule prior to surgery and confirms indirectly the implication of the partial laryngectomy.

CONCLUSIONS

1. A cervical mass occurring a few years after a subtotal laryngectomy is not always a cervical or laryngeal recurrence.

2. This surgical procedure increases the risk of developing an iatrogenic laryngocele, with an incidence of 2% in our experience. The recent improvement of these surgical procedures will probably increase the incidence of these complications. The obstruction of the mouth of the saccule is likely to produce an iatrogenic mucocele, as in our patient.

3. The diagnosis of a coexisting laryngocele with cancer has to be made before the surgical procedure with the help of a CT scan, to allow radical excision of the dilated saccule in conjunction with partial laryngectomy and to prevent inadequate and incomplete resection. In the same way, an exploration and exeresis of a normal saccule seems to be required when a concomitant excision of the ventricle or anterior part of the false vocal cord in association with the glottic level is performed.

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TRAUMATOLOGY OF THE HEAD AND NECK

The Berlin Course on Traumatology of the Head and Neck (with osteosynthesis exercises and live operation) will be held November 5-7, 1998, in Berlin, Germany. The special topic will be acceleration-deceleration trauma (whiplash). For information, contact Prof Arne Ernst, Dept of Otolaryngology at UKB, Rapsweg 55, D-12683 Berlin, Germany; telephone 030 5681 2801; fax 030 5681 2903; e-mail ernst.orl.ukberlin@t-online.de.

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CERVICAL THOROTRAST GRANULOMA: A REVIEW OF THE LITERATURE AND A NEW TREATMENT MODALITY

FEODOR UNG, MD
JOHN B. LAZOR, MD
WILLIAM W. MONTGOMERY, MD
BOSTON, MASSACHUSETTS

Extravasation of thorium dioxide after transcervical carotid angiography has resulted in persistent open draining neck wounds. These difficult problems have remained a challenge for the treating head and neck surgeon. Neck dissection has been the mainstay of treatment in the past; however, this has been fraught with complications. The application of doxycycline sclerosis is described in the successful resolution of a large Thorotrast granulomatous neck wound. A review of the literature and the management options of Thorotrast granulomas are discussed.

KEY WORDS — contrast medium complications, doxycycline sclerosis, neck, thorium dioxide, Thorotrast, thorotrastoma, wound healing.

INTRODUCTION

Thorotrast is a suspension of thorium dioxide that was concocted by Radt in 1928.[1] It was used extensively in diagnostic radiology from the 1930s to the 1950s,[2,3] until reports of its carcinogenic and fibrogenic effects led to its proscription. Before its ban, hundreds of patients received Thorotrast during transcervical carotid angiography. Extravasation of contrast material during the procedure produces a fibrotic foreign body reaction resulting in a Thorotrast granuloma. Over the course of decades, these patients may experience vocal cord paresis, mass effects, deficits of cranial nerves IX through XII, dyspnea, dysphagia, odynophagia, and cerebrovascular insufficiency.[1,2,4-6] A neck dissection with excision of the granuloma has been the treatment of choice in the past; however, serious complications such as carotid rupture[6-8] and mediastinitis[3,4,9] have precluded this as a primary form of intervention. We describe the application of doxycycline as a sclerosing agent used to successfully heal a chronic draining Thorotrast granuloma cavity.

CASE REPORT

The patient is a 74-year-old man who was referred to our institution in 1987 for evaluation of a right vocal cord paralysis. Fiberoptic examination of the larynx confirmed the presence of a right vocal cord paralysis. This vocal cord paralysis was attributed to entrapment of the vagus or recurrent laryngeal nerve by a right-sided Thorotrast granuloma.

The patient's history began in 1943 while he was a member of the US armed forces fighting in World War II. At that time, he sustained a head injury and suffered post-traumatic seizures. For evaluation of the seizures, he underwent a right carotid angiogram employing Thorotrast. Extravasation of the contrast material during the procedure led to the formation of a right cervical Thorotrast granuloma. The presence of this right neck mass was later confirmed in 1964 during an unrelated admission for thyrotoxicosis.

Since his referral to our institution in 1987, the patient has been followed closely. In 1990, carotid Doppler studies demonstrated adequate cerebrovascular circulation with only a 20% occlusion of the left internal carotid artery. Aside from complaints of hoarseness and dysphagia, the patient did well until 1992, when he was found to have retroperitoneal lymphoma after a routine physical examination. He was treated with chemotherapy and has since remained disease-free. Of interest, it was noted that during the patient's chemotherapy, the size of his neck mass decreased. Afterward, the patient continued to complain of dysphagia and aspiration symptoms. Further evaluation revealed evidence of bulbar myasthenia gravis, and the patient was treated with pyridostigmine bromide and prednisone with some amelioration of symptoms. However, the patient continued to display no improvement in his voice quality, and the vocal cord remained in a paramedian position. A right medialization laryngoplasty was considered because of the patient's aspiration symptoms and poor voice quality. Preoperative computed tomography (CT) scans indicated that the thyroid cartilage was distinct and separate from the Thorotrast granuloma site (see Figure, A). In April of

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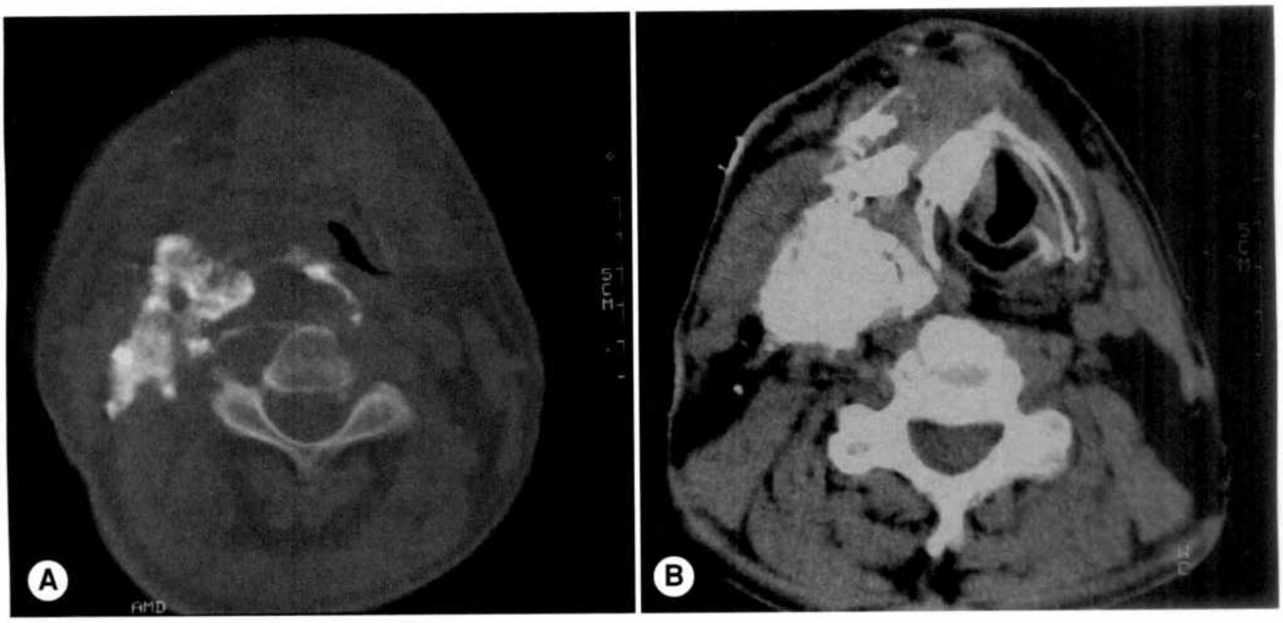
From the Department of Otolaryngology, Massachusetts Eye and Ear Infirmary, and the Department of Otology and Laryngology, Harvard Medical School, Boston, Massachusetts.

CORRESPONDENCE — William W. Montgomery, MD, Massachusetts Eye and Ear Infirmary, 243 Charles St, Boston, MA 02114.



Computed tomograms. A) Extensive Thorotrast infiltrating soft tissues of right side of neck. Airway compromise was present secondary to Thorotrast mass infiltrating deep neck spaces. B) At time of right neck hemorrhage, revealing thyroplasty prosthesis in good position.

1995, the patient proceeded to surgery, and a right medialization laryngoplasty was performed. During the procedure a 5-cm cystic area was encountered, the contents of which were drained and sent for serology. No cells were noted in the fluid at that time. Postoperatively, a thick, purulent discharge flowed continuously from this cavity, but closure was eventually achieved.

The patient did well until July of 1995, when he experienced a swelling in the right neck region and was taken back to the operating room. Exploration of the right side of the neck revealed that the cystic area was filled with thick secretions. Penrose drains were placed in the cavity, which persistently drained thick secretions. The patient was followed up weekly; however, the drainage failed to resolve. In September of 1995, a course of doxycycline to sclerose the nonhealing Thorotrast cavity was begun. Iodoform gauze soaked in a doxycycline solution (100 mg in 10 mL of normal saline) was packed into the cavity and changed on a weekly basis.

In October, the patient experienced an episode of brisk bleeding from his neck wound. After control of the hemorrhage was achieved, a CT scan and an angiogram were obtained. These tests demonstrated a 4-mm pseudoaneurysm of the right common carotid artery bulging into the cystic cavity. The Montgomery thyroplasty prosthesis was noted to be in good position (see Figure, B). Interventional radiology determined that embolization was necessary to prevent further bleeding. Three balloons were placed without neurologic sequelae, and the patient subsequently experienced no further hemorrhagic events. He was followed closely and continued to receive doxycycline sclerosing sessions on a weekly basis. Over the ensuing 36 weeks, the cavity closed from 5 cm to less than 0.5 cm. The patient is currently healed. He has no dysphagia or dysphonia.

DISCUSSION

Thorotrast is a colloidal suspension of thorium dioxide in a dextran solution that was once utilized as a contrast medium in diagnostic radiology.[2,8,10] It enjoyed widespread use after its introduction in the late 1920s and continued to be administered as late as 1958.[8] It was virtually the only contrast medium in use prior to 1945[11] and was routinely employed in intravascular, intracavitary, and interstitial imaging.[12,13] Its popularity as an intravascular agent stemmed from its high atomic density, which produced excellent contrast, and its painless injection without resulting vasospasm.[8,10] As many as 100,000 people worldwide and 4,300 in the United States were imaged with Thorotrast before evidence of complications led to its prohibition.[1,2,6]

The radioactive agent in Thorotrast, thorium dioxide, has a physical half-life of 1.39×10^{10} years and a biological half-life of 400 years.[7,9,13,14] Once injected into the body, it becomes permanently deposited there because of its long half-life and negligible excretion.[6,8,12] The decay of thorium emits 90% alpha, 9% beta, and 1% gamma radiation.[6-8,10] These emissions actually increase with time, due to the higher radioactivity of thorium's daughter isotopes,[3] reaching peak radiation 30 years after administration.[8] Since thorium is actively phagocytosed from the bloodstream by macrophages, it is accumulated after intravascular administration by the liver, spleen, bone, and other organs of the reticuloendothelial system.[2,9,13] Other areas of deposition are regions around injection sites into which Thorotrast may have extravasated or hollow cavities in which Thorotrast was instilled and is now retained.[10,13] Tissues surrounding these Thorotrast deposits are constantly bombarded by particles released by the compound.

Concern over the radioactive properties of Thorotrast led the Council of Pharmacy and Chemistry of the American Medical Association to condemn its use in 1932, although it continued to be used in the United States as late as 1954.[3,6,15,16] France prohibited the use of Thorotrast in 1935,[10,15] after researchers there discovered that the contrast agent induced sarcomas in laboratory animals.[3,6,16] By 1947, fears regarding the hazardous nature of Thorotrast were realized with the description of the first fatality related to its use: a case of hepatic angiosarcoma occurring 12 years after intrahepatic injection.[10,16,17] Over the years, epidemiologic studies have correlated Thorotrast exposure to a greatly increased incidence of liver cancer, hepatoma, leukemia, and liver cirrhosis.[11,18] Andersson et al[11] noted a 50% excess mortality in patients exposed to Thorotrast, mainly due to liver disease.

The iatrogenic sequelae from Thorotrast injection fall into two categories of cellular injury: neoplasia and fibrosis. Thorotrast has been directly implicated in carcinogenesis, with those exposed being two to three times more likely to develop cancer.[11] The most common sites of neoplastic transformation are the liver, spleen, and bone areas, which accumulate the most Thorotrast. As a consequence, the most common neoplasms are hepatic angiosarcoma, cholangiocarcinoma, and blood dyscrasias.[1,2,8] Besides its oncogenic effects, Thorotrast incites a dense foreign body reaction that obscures and destroys normal tissue planes. Grossly, these areas of fibrosis are chalky white, partly calcified, and tenaciously adherent to surrounding structures.[3,14] Soft, necrotic regions may be encountered within this reactive tissue.[8,19] Microscopically, the fibrotic masses consist of dense, hypocellular connective tissue with macrophages, giant cells, and lymphocytes surrounding gray-brown Thorotrast granules.[5,6,8,10]

The head and neck surgeon should be familiar with the unique manifestations of Thorotrast-induced cellular injury in the head and neck region. Neoplastic disease usually takes the form of sinonasal carcinoma. Tumors of the maxillary sinus can result from intracavitary instillation of Thorotrast to outline the sinonasal region. These tumors are extremely rare, with only 18 reported cases,[8] the majority of which were squamous cell or mucoepidermoid carcinoma.[6] Far more commonly encountered than sinonasal neoplasia is the Thorotrast granuloma, a fibrotic reaction caused by the extravasation of Thorotrast during transcervical carotid angiography. Of the thousands who received Thorotrast carotid angiography, 3% to 10% experienced extravasation during administration, with 50% to 60% eventually developing granulomas.[1,3,8] Malignant degeneration associated with these masses has been described in 13 cases.[6,9]

The diagnosis of Thorotrast granuloma can be made on the basis of an accurate history and physical examination. However, given a latency that can span decades, precise documentation of Thorotrast administration may not be obtained. Therefore, the physician should have a high index of suspicion in anyone who received contrast for diagnostic radiology between the 1930s and 1950s.[7] Plain films of the neck and abdomen can greatly contribute to the diagnosis. Neck films and CT scans characteristically demonstrate a unilateral radiopacity of metallic density that can extend from the base of the skull to the mediastinum, roughly along the course of the carotid artery.[1,6] The irregular, streaky densities do not conform to any body cavity, but may dissect along fascial planes.[17,20] Progressive widening of these fascial planes, along with increased nodularity of the lesion, may be seen on serial films.[19] The diagnosis may be confirmed by an abdominal series, in which diffuse, punctate, or reticulate opacification of the liver and spleen, in conjunction with extremely dense opacification of the abdominal lymph nodes, is virtually diagnostic of Thorotrast administration.[6,9,20,21] Adjunctive diagnostic modalities include histologic studies and autoradiography of biopsy specimens.[6,10,21]

The latent period before the development of symptoms is 20 to 30 years,[3,6] consistent with the increasing radioactivity of thorium and the ensuing slow destruction of tissue planes over time. As was the case with our patient, the two most common presentations in the head and neck are a palpable mass and vocal cord paralysis.[6] Mass effects may produce obstructive symptoms such as dyspnea, dysphagia, and cerebrovascular insufficiency.[4,6,14] The infiltrative nature of the lesion may cause pain and torticollis as the cervical anatomy is invaded.[4,6] Nerves are particularly susceptible to infiltration, with irreversible damage caused by the progressive demyelination and subsequent fibrosis.[3,4,12] Palsies of cranial nerves IX to XII may result, contributing to dysphagia, dyspnea, hoarseness, aspiration, and tongue atrophy.[1,2,14] The sympathetic chain may also be involved by the granuloma, producing Horner's syndrome.[1,14] In addition, vascular structures are prone to Thorotrast-induced injury. Besides the compressive and obstructive effects of the granuloma, Thorotrast radiation damages the microcirculation of vessels, producing an avascular necrosis.[8] A contributing factor in vascular injury may be the atherogenic properties of radiation, which produces severe intimal thickening.[17,18,22] The poor healing capabilities caused by the impaired microcirculation promote mucosal breakdown and the development of pharyngocutaneous fistulae.[4] It should be noted that transoral biopsy is contraindicated due to the high incidence of infection with resultant fistula formation at the biopsy site.[2-4,7] Late sequelae of vascular degeneration are cerebrovascular insufficiency, thromboembolism, vessel erosion, and rupture.[1,2,4,6]

The rationale for treatment has slowly evolved since the late 1940s, when Amory and Bunch performed the first modified neck dissections to treat Thorotrast granulomas. Of their initial two patients, one failed to be relieved of his symptoms and the other developed a rapid recurrence of the granuloma.[12] Other authors have noted poor wound healing, fistula formation, and inadequate removal of radioactive material after partial resection.[23] A more extensive approach to the disease was deemed necessary, and in 1960, the first radical neck dissection of a Thorotrast granuloma was reported. An uneventful recovery with significant and lasting improvement of symptoms was described.[16] However, as more cases were treated with radical neck dissection, devastating and often fatal complications such as carotid artery rupture, pharyngeal necrosis, and descending mediastinitis were reported.[9,13] Stanley and Calcaterra,[3] in their series of Thorotrast granulomas treated with radical neck dissection, found a correlation between carotid obstruction and increased survival due to the high incidence of carotid rupture after radical neck dissection. Secondary to the potentially fatal complications, radical neck dissection has fallen out of favor and is no longer the treatment of choice.

Currently, the indications for surgery remain unclear. It has been the experience of the senior author (W.W.M.) that the surgeon must weigh the risk of surgery versus the local effects and the risk of malignancy. Surgery may retard or stop the progression of local symptoms while improving respiratory function, swallowing, and appearance.[3,4] In addition, debulking the granuloma may decrease the radiation load in the neck and thus decrease the risk of sarcomatous transformation. However, the incidence of such transformation is rare, and even the most heroic surgical procedures will leave some residual Thorotrast due to the extensive and tenacious nature of the lesion. In addition, radical neck dissection would not decrease the risk of a reticuloendothelial malignancy, which is far more likely. Overall, surgical survival is poor and is frequently complicated by poor wound healing and infection, which may progress to the more disastrous developments outlined above. In light of the surgical hazards, the senior author supports conservative management, but does not rule out surgical intervention on or around these lesions.

In the case described, CT scans demonstrated a clear separation between the thyroid cartilage and the granuloma, and it was believed that a medialization laryngoplasty could be achieved without violating the lesion. Given the age, loss of voice, and aspiration symptoms of the patient, surgery was indicated. The postoperative period was plagued by poor wound healing, as are many procedures in previously irradiated fields. Wet-to-dry dressing changes were used for many weeks without improvement. Aware of the disastrous consequences of wound breakdown, the senior author elected to treat the open wound with doxycycline sclerosis. The rationale for this treatment was based on his previous success in treating cystic hygromas and other head and neck disorders with the same regimen. The excellent results obtained in our case may provide head and neck surgeons with an additional treatment modality when faced with poorly healing wounds from operating in or around a Thorotrast granuloma.

SUMMARY

Thorotrast granulomas present the head and neck surgeon with chronic debilitating problems and few successful solutions. Doxycycline sclerosis has been used successfully in the treatment of recurrent cystic hygromas. Its application in the treatment of a chronically draining neck Thorotrast granuloma proved successful after 36 weeks of intensive therapy. This may prove to be beneficial for similar patients with this difficult problem.

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CYTOKINE EVALUATION IN THROAT INFECTIONS

ELIMELECH DEUTSCH, MD
BENJAMIN NISMAN, MD, PHD
MICHAL KAUFMAN, MD
VIVIAN BARAK, PHD
JERUSALEM, ISRAEL

Inflammatory cytokines have been shown to play an important role in the pathogenesis of various inflammatory processes. In throat infections, intracellular inflammatory cytokines have been detected from the sites of inflammation. The present study aimed to evaluate serum cytokine levels of patients with throat infections and correlate them to the inflammatory parameters and type of inflammation. Significantly higher inflammatory cytokine levels (interleukin [IL]–6 >7 pg/mL, IL-1 >1ß pg/mL, tumor necrosis factor α >1 pg/mL) were detected in most of the patients as opposed to healthy controls. Clinical parameters of infection (fever >38°C, leukocytosis >11,000 white blood cells per cubic millimeter, polymorphonuclear neutrophils >75%) were significantly correlated with high levels of inflammatory cytokines: mainly IL-6 and tumor necrosis factor α, and to a lesser degree with IL-1ß. No correlation, however, was found between the type of inflammation and cytokine levels. The present study indicates a role of inflammatory cytokines in the pathogenesis of throat infections and the need for an anti-inflammatory and anticytokine therapeutic approach.

KEY WORDS — cytokines, inflammation, throat infections.

INTRODUCTION

There is an increasing body of evidence that cytokines play a significant role in the pathogenesis of inflammation. Allergic inflammation is characterized by the presence of eosinophils, lymphocytes, mast cells, and basophils. Many of these cells have been shown to produce various cytokines. Cytokines have been shown to be crucial in the regulation of immune and inflammatory responses in adhesion, migration, proliferation, and activation of various cells. Cytokine secretion is known today to contribute also to the activation of and effects on the hormonal and neural systems.[1-3]

Cytokines can be broadly classified into two groups. Proinflammatory cytokines, primarily produced by activated macrophages, but also by many additional types of cells, are interleukin (IL)–1, IL-6, IL-8, interferons (IFNs), and tumor necrosis factor α (TNF-α). The second group includes growth factor cytokines (IL-2, IL-3, IL-4, IL-5, IL-7, IL-10, and colony-stimulating factors). Considerable overlap in their function may exist.[4-7]

Cytokines have been shown to have a crucial role in the pathogenesis of inflammatory processes in the ear, nose, and throat system.[8-11] Some cytokines were recovered from the sites of pathologic processes. Inflammatory cytokines have also been shown in cholesteatoma tissue.[12] Increased IL-1 activity in cholesteatoma epithelium and stroma correlated significantly with the presence of bone erosion.[13] Intracellular inflammatory cytokines were also detected from tonsillar tissues in children with recurrent tonsillitis or infectious mononucleosis.[14]

In middle ear effusions, high levels of the cytokines IL-1α, IL-1ß, IL-2, TNF-α, IFN-γ, and IL-6 in serum or cells were previously shown.[10,15] Indomethacin, known to inhibit prostaglandin E2 (PGE2) and cytokine production, was shown to inhibit middle ear bone resorption as well, probably because of cytokine effects.[16]

In nasal polyposis, TNF-α was found to be of pathogenetic importance,[17] and in frontoethmoid mucocele, IL-1ß, IL-6, TNF-α, and IL-8 were suggested to have a role in bone destruction processes.[18]

Aiming to better understand and possibly identify factors influencing the pathogenesis of throat infections, we evaluated the main inflammatory cytokine levels (IL-1ß, IL-6, TNF-α) in patients' blood and correlated them to the degree of the clinical parameters of throat infection. We also tried to find correlations between the type of regional inflammation (tonsillitis, peritonsillitis, peritonsillar abscess) and cytokine levels.

METHODS

The 30 patients included in this study (17 female, 13 male, age range 12 to 50 years, mean age 31) came to our emergency room with general symptoms like fever or malaise originating from throat infections. After diagnosis, specific cultures were taken from the sites of infection and blood samples were drawn for complete blood count, electrolytes, and cytokines. Most of the patients were seriously ill and required intravenous antibiotic treatment, as

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From the Department of Otorhinolaryngology, Bikur Holim Hospital (Deutsch, Kaufman), and the Immune Laboratory for Tumor Diagnosis, Hadassah University Hospital (Nisman, Barak), Jerusalem, Israel.

CORRESPONDENCE — Elimelech Deutsch, MD, Dept of Otorhinolaryngology, Bikur Holim Hospital, Jerusalem, Israel.

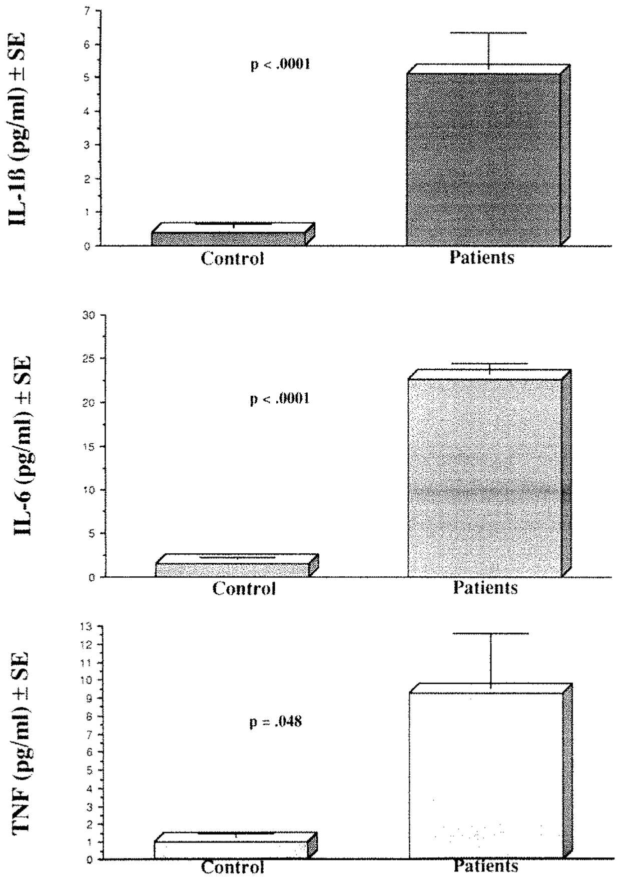

Fig 1. Elevated cytokine levels in patients' sera. IL — interleukin, SE — standard error, TNF — tumor necrosis factor α.

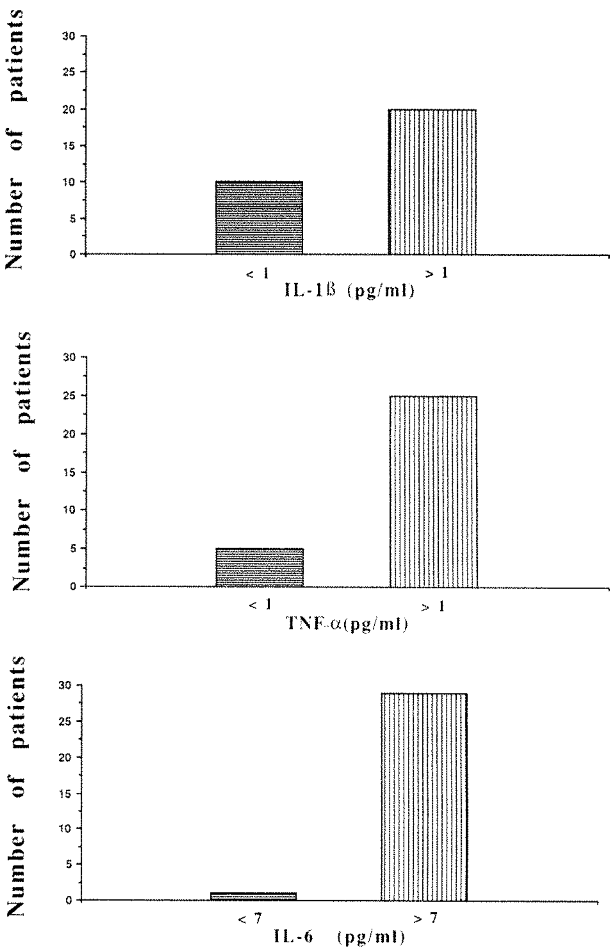

Fig 2. Number of patients with elevated cytokine levels.

well as treatment with fluids, antipyretics, and analgesics.

Blood samples were centrifuged and the sera kept frozen (–70°C) until assayed for cytokines.

Cytokine Evaluation. Serum levels of cytokines (IL-1ß, IL-6, and TNF-α) were measured by highly sensitive enzyme-linked immunosorbent assay (ELISA) kits (Quantikine HS). The ELISA kits were purchased from Research and Diagnostic Systems (Minneapolis, Minn). These kits use an amplification system in which the alkaline phosphatase reaction provides a cofactor that activates a redox cycle leading to the formation of a colored product. The secondary enzyme system consists of alcohol dehydrogenase and diaphorase (amplifier). In general, the ELISA employs the quantitative "sandwich" enzyme immunoassay technique. A monoclonal antibody specific for the interleukin molecule has been precoated onto the polystyrene microtiter plate. Standards and samples are introduced into the wells, and the interleukin present is bound by the immobilized antibody. After washing away any unbound proteins, the second enzyme-linked polyclonal or monoclonal antibody specific for the interleukin is added to the wells to "sandwich" the interleukin immobilized during the first incubation. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of interleukin bound in the initial step. The color development is stopped and the intensity of the color is measured. A curve is prepared, plotting the optical density versus the concentration of the given interleukin in the standard wells. By comparing the optical density of the samples to this standard curve, the concentration of the interleukin in unknown samples is then determined.

RESULTS

Elevated Cytokine Levels in Patients. All patients were evaluated clinically and their cytokine levels were determined. Levels of IL-6 >7 pg/mL, IL-1ß >1 pg/mL, and TNF-α >1 pg/mL were defined as high levels when compared to a group of 25 healthy controls (Fig 1), described by us elsewhere.[19,20] Elevated IL-6 was found in all patients but 1 (96.6%), and elevated IL-1ß (20/30 or 66%) and TNF-α (25/30 or 83%) levels were present in the majority of patients (Fig 2).

CORRELATION BETWEEN ELEVATED CYTOKINE LEVELS AND CLINICAL PARAMETERS OF INFECTION

	Clinical Laboratory Parameters			
Cytokines	*Fever* $\geq 38°C$	*Leukocytosis* >*11,000 WBC/mm*^{3}	*PMN* >*75%*	*Combined*
IL-6 (>7 pg/mL)	100.0%	95.2%	100.0%	100.0%
IL-1β (>1 pg/mL)	66.0%	52.4%	71.4%	60.0%
TNF-α (>1 pg/mL)	89.0%	81.0%	76.0%	100.0%

Percentages are of patients.

WBC — white blood cells; PMN — polymorphonuclear neutrophils; combined — fever ≥38°C + leukocytosis >11,000 WBC/mm^3 + PMN >75%; IL — interleukin; TNF-α — tumor necrosis factor α.

Correlations Between Elevated Cytokine Levels and Inflammatory Parameters. Correlations between elevated cytokine levels and each elevated inflammatory parameter or combined elevated inflammatory parameters were performed and are presented in the Table. Significantly elevated inflammatory cytokine serum levels were found in the majority of our patients (IL-1ß $p < .0001$, IL-6 $p < .0001$, TNF-α $p = .048$; Fig 1).

High fever was associated with high levels of IL-6 and TNF-α in most of the patients and with high levels of IL-1ß in some of the patients. Leukocytosis (>11,000 white blood cells per cubic millimeter) was also correlated in most of the patients with high IL-6 and TNF-α levels, and in some patients also with high IL-1ß levels. Polymorphonuclear neutrophils (>75%) were mostly correlated with high IL-6 and TNF-α levels, and only partly with high IL-1ß levels. When the three inflammatory parameters were combined, a complete and significant correlation was shown with high IL-6 and TNF-α levels, and a partial correlation with high IL-1ß levels (see Table).

Correlations Between Elevated Cytokine Levels and Types of Infection. The patients were classified according to the type of infection into three groups (5 patients with peritonsillar abscess, 15 patients with acute tonsillitis, and 10 patients with peritonsillitis), and cytokine levels were correlated to these groups. In the two large groups of patients, the trend of cytokine levels was very similar to that of the whole study population. The first group was very small (5 patients); therefore, no conclusions can be drawn. In the peritonsillar abscess group (5 patients), 100% of patients had IL-6 levels of >7 pg/mL, 60% had IL-1ß of >1 pg/mL, and 60% had TNF-α of >1 pg/mL. In the acute tonsillitis group (15 patients), 94% had IL-6 of >7 pg/mL, 62% had IL-1ß of >1 pg/mL, and 87% had TNF-α of >1 pg/mL. In the peritonsillitis group (10 patients), 100% had IL-6 of >7 pg/mL, 70% had IL-1ß of >1 pg/mL, and 90% had TNF-α of >1 pg/mL.

DISCUSSION

The biological activities of cytokines imply that they participate in both restorative and destructive processes that follow infection or injury. Detection of cytokines in body fluids or tissues may be useful in diagnosis of inflammatory conditions, or in evaluation and monitoring of drug effects in the upregulation or downregulation of an inflammatory state.

In tonsillar tissues, intracellular inflammatory cytokines have already been described.[14] Interleukin-1ß, TNF-α, and IL-6 have been shown both in human fluids or cells[11,12] and in experimental conditions of otitis media.[9-12]

Our study is the first (to the best of our knowledge) to evaluate blood cytokine levels during acute tonsillitis with regional damage and systemic effects and to correlate them with the disease severity. Another study showed cytokines originating from tonsillar tissues.[14] Similar to other acute infectious states,[4,6,18] we have shown that in our patients with acute tonsillitis, blood levels of inflammatory cytokines were significantly higher as compared to those in healthy volunteers ($p < .01$). These high cytokine levels were correlated significantly with systemic inflammatory parameters such as fever, leukocytosis, and polymorphonuclear neutrophils, with the highest correlation to IL-6 and a lesser correlation to TNF-α and IL-1ß. No correlation between the type of regional inflammation and elevated cytokine levels was observed. We have to take into consideration that most of the patients were hospitalized with severe conditions, some being treated with antibiotics, and so most of their blood cultures were negative. This clinical picture indicates that the regional and systemic damage has been induced primarily by bacteria and/or viruses and secondarily by the inflammatory cytokine cascade developed and induced by the primary stimulus and the cytokines themselves.

The present study revealed that the inflammatory state of throat infections is significantly correlated with high inflammatory levels of cytokines detected in sera of patients, which might contribute to the evolution of throat infections and to the infiltration of inflammatory cells. This finding of a correlation between the severity of the infection and levels of IL-6, TNF-α, and IL-1ß raises the question of whether cytokine tests could affect the choice of antibiotic treatment. Prolonged retention of throat infections might be induced by the persistent presence of inflammatory cytokines. An anti-cytokine approach consisting of antibodies to cytokines or to cytokine receptors, soluble cytokine receptors, or cytokine antagonists might be an alternative or adjuvant therapeutic approach.

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ISOLATED EPITHELIAL CYSTS OF THE UVULA IN CHILDREN: A REPORT OF THREE CASES

MERRITT SESHUL, MD
BIRMINGHAM, ALABAMA

EWEN TSENG, MD
PLANO, TEXAS

BRIAN J. WIATRAK, MD
BIRMINGHAM, ALABAMA

DAVID R. KELLY, MD
BIRMINGHAM, ALABAMA

Epidermoid cysts are unusual benign cysts that occur in the oral cavity less than 2% of the time. Epidermoid cysts isolated to the uvula are rare. A review of the literature has revealed only three confirmed cases of isolated uvular epidermoid cysts. We present a series of three such cases, two of which presented with significant symptomatology. The diagnosis and management of these unusual lesions will be discussed, as well as the differential diagnosis.

KEY WORDS — epidermoid cyst, epithelial cyst, uvula.

INTRODUCTION

Epidermoid cysts are benign cystic lesions that are lined with a layer of epithelium without any adnexal structures. These cysts can occur anywhere in the body, although 7.0% of all epidermoid cysts occur in the head and neck area and only about 1.6% occur in the oral cavity.[1,2] Most epidermoid cysts of the oral cavity occur in the anterior floor of the mouth.[3] Various cysts can occur in the soft palate and the region of the uvula.[3,4] However, isolated epidermoid cysts of the uvula are rare, with only three individual cases having been reported.[5,6] We present a series of three more cases of isolated epidermoid cysts of the uvula.

CASE REPORTS

Case 1. A 1-year-old boy presented with excessively loud snoring. Physical examination revealed a 1.0 × 1.0-cm submucosal cyst within the uvula (Fig 1A). Surgical resection was performed, and histologic examination revealed a benign cyst with squamous epithelial lining (Fig 1B). The patient did well postoperatively, with resolution of the snoring.

Case 2. A 1-month-old boy presented with an asymptomatic mass of the uvula. On physical examination, the mass initially measured about 0.5 cm in diameter within the uvula (Fig 2A). As the lesion continued to grow, the patient began to experience

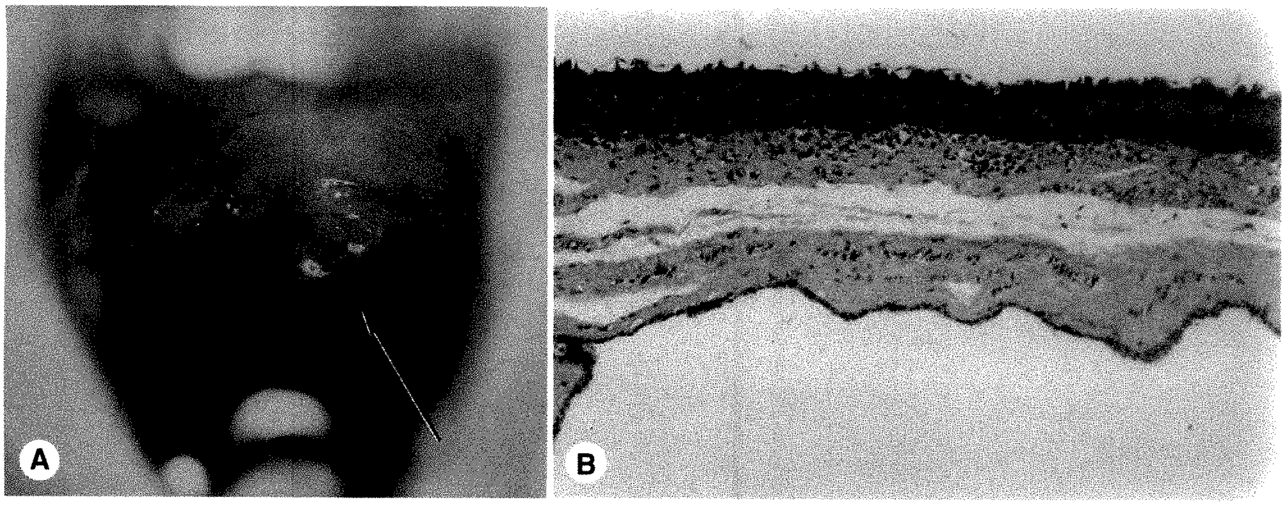

Fig 1. (Case 1) A) Intraoperative examination reveals 1.0 × 1.0-cm submucosal cyst (arrow) within uvula. B) Cyst lined by simple cuboidal to columnar epithelium (original ×140).

From the Departments of Pediatric Otolaryngology–Head and Neck Surgery (Seshul, Tseng, Wiatrak) and Pathology (Kelly), Children's Hospital, University of Alabama at Birmingham, Birmingham, Alabama.

CORRESPONDENCE — Brian J. Wiatrak, MD, Dept of Pediatric Otolaryngology–Head and Neck Surgery, Children's Hospital, 1600 Seventh Ave S, ACC 320, Birmingham, AL 35233.

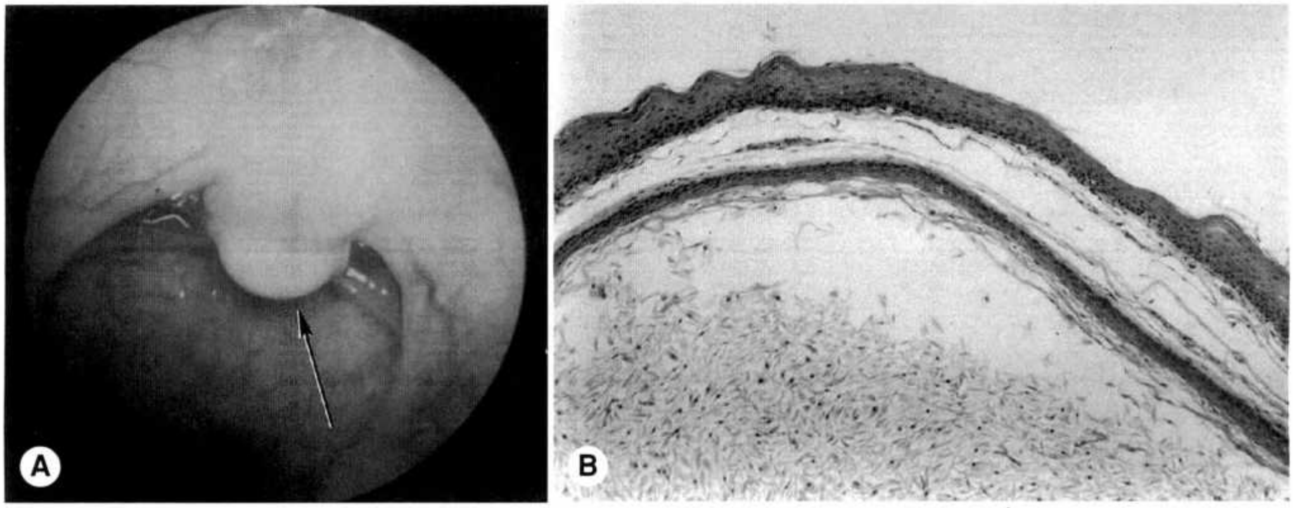

Fig 2. (Case 2) A) Intraoperative examination reveals 0.5-cm mass (arrow) in uvula. B) Small cyst filled with squames and lined by nonkeratinizing stratified squamous epithelium (original ×140).

gagging, dysphagia, and snoring. The cyst was surgically resected. Histopathology revealed a cyst filled with sebaceous material and lined with a nonkeratinizing stratified squamous epithelium (Fig 2B). The patient had an uneventful postoperative course, with no further problems with feeding or snoring.

Case 3. A 3-month-old boy presented with a low-grade fever, irritability, and drooling. Plain radiographs of the airway showed thickening of the retropharyngeal soft tissue. No evidence of gas or air-fluid levels were noted, and there was a normal epiglottis. The white blood cell count was mildly elevated. Some purulent material was noted along the posterior pharyngeal wall. The patient was intubated to protect his airway and transferred to the hospital. A computed tomography scan of the neck revealed a normal posterior pharyngeal wall. The only abnormality noted was a cystic lesion at the level of the soft palate. Given the patient's history, he was taken for an examination under anesthesia and direct laryngoscopy. At the time of surgery, a 0.5-cm white, cystic lesion was found within the uvula (Fig 3A). The lesion was surgically resected. The oropharynx was slightly hyperemic but otherwise normal. The larynx was normal. Histopathology revealed an epithelial cyst of the uvula (Fig 3B). The patient was then extubated and taken to the postanesthesia recovery unit in good condition. Over the next 2 days, the patient's low-grade fever resolved and the white blood cell count returned to normal. Further investigation concluded that the patient had previously been asymptomatic from the lesion. It was determined that the patient had had a viral pharyngitis with an incidental finding of a uvular epidermoid cyst.

DISCUSSION

Isolated cysts of the uvula and soft palate are un-

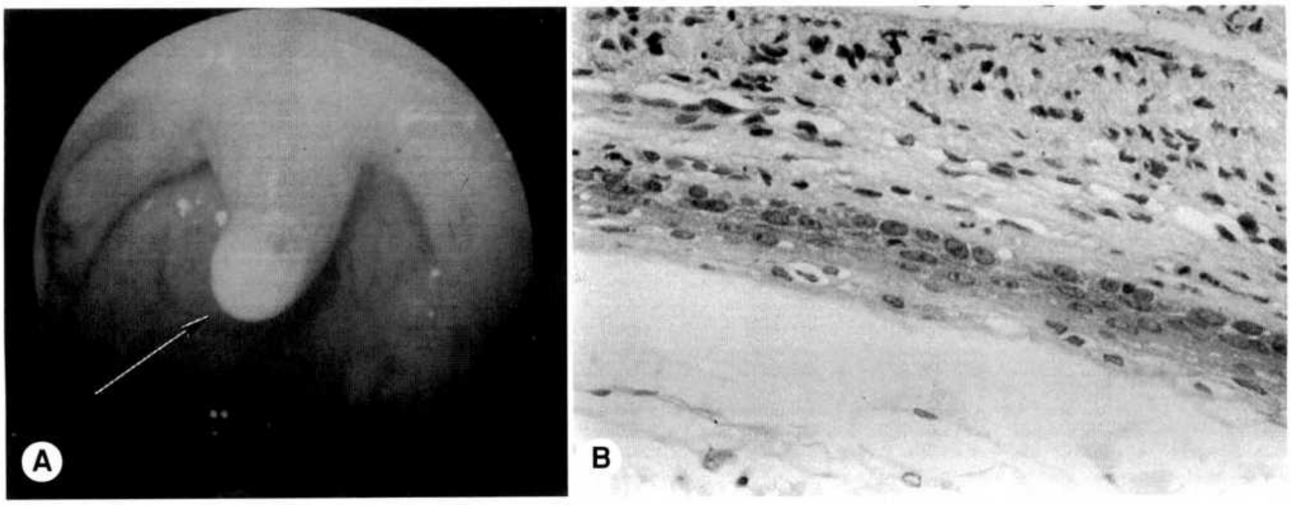

Fig 3. (Case 3) A) Intraoperative view of 0.5-cm cystic lesion (arrow) within uvula. B) Cyst lined by stratified squamous epithelium (original ×560). Minimal mucous debris, and lymphocytic infiltrate in surrounding soft tissues.

common. Reported lesions include 1) branchial cleft cyst,[7] 2) dermoid cyst,[3] 3) teratoma cyst,[8] and 4) inclusion cyst as a result of uvulopalatopharyngoplasty.[4] The reported isolated epithelial cysts of the uvula in children were variably symptomatic.[5,6] One of these lesions partially obstructed the nasopharynx in a newborn infant. Of interest, on review of our own experience, we encountered one case of a 20-month-old boy, 15 months status post resection of a right-sided, second branchial cleft cyst, who presented with snoring and a rapidly enlarging right-sided uvular cyst. The histopathology of this lesion after resection revealed a mucocele. Mucoceles, unlike epithelial cysts, are not lined with a true layer of squamous epithelium.

Epidermoids of the oral cavity and submental area are likely to arise from ectodermal sequestration during the midline closure of first and second branchial arch derivatives. Fusion of the lateral processes of the first branchial arch can also produce these epidermoid cysts. Specifically, fusion of the palatine plates can give rise to epidermoid cysts in the area of the soft palate and uvula.[3,5]

Patients with uvular cysts can have a variable clinical presentation: asymptomatic (case 3), with snoring (cases 1 and 2), or with significant gagging and dysphagia (case 2). In evaluation of cystic lesions in the region of the uvula, the differential diagnosis should include dermoids, teratomas, mucoceles, inclusion cysts, and branchial cleft cysts. Excisional biopsies are both diagnostic and curative, and the procedure is usually well tolerated.

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MAJOR SURGICAL COMPLICATIONS

CLAVICULAR OSTEOMYELITIS AS A COMPLICATION OF HEAD AND NECK SURGERY

CATHERINE P. WINSLOW, MD ARLEN D. MEYERS, MD, MBA

DENVER, COLORADO

INTRODUCTION

Clavicular osteomyelitis is a rare but important entity that should be familiar to all head and neck surgeons as a potential complication. Prevention and early diagnosis will lead to successful outcomes. Aggressive surgical attention is of primary importance, and appropriate antibiotic therapy will assist in complete recovery. A 10-year retrospective chart review was performed at 2 major medical centers (local university and Veterans Administration hospitals) to determine the incidence and outcome of clavicular osteomyelitis — in particular, those cases caused by head and neck surgery. Four cases of clavicular osteomyelitis were identified; 2 of these were attributed to antecedent head and neck surgery.

CASE REPORTS

Case 1. A 60-year-old man was seen at the University of Colorado hospital with a T2N0M0 supraglottic squamous cell carcinoma and underwent total laryngectomy in March 1993. The patient had postoperative radiotherapy, and was noted to be hypothyroid following this. He was placed on thyroid replacement and did well until he developed symptomatic stomal stenosis in June 1996. He underwent stomatoplasty in July 1996 with an inferiorly based flap. He subsequently developed a pharyngocutaneous fistula, which had not been noted in surgery. The fistula was managed conservatively, and the patient was discharged with a nasogastric tube.

The patient failed to return for scheduled appointments. He appeared in the emergency department (ED) on August 31, 1996, with fever to 40°C (104°F), diarrhea for 3 days, and pain at the stoma and right shoulder, which the patient stated had worsened in the preceding 3 days. Examination revealed a temperature of 38.9°C (102°F), erythema, inflammation, and tenderness over the inferior stoma and extending to the right superior thorax. The pharyngocutaneous fistula was still patent. The patient was given intravenous vancomycin hydrochloride in the ED, refused admission, and left against medical advice. The following day he returned to the ED and allowed himself to be admitted. Intravenous antibiotics were administered and a chest radiograph was obtained, which was suspicious for right pneumonia. A computed tomography (CT) scan revealed a right sternoclavicular joint abscess and was suspicious for mediastinitis (Fig 1). The patient underwent debridement of the sternoclavicular joint September 6, 1996. The patient did well postoperatively with no further problems, and left against medical advice on September 12.

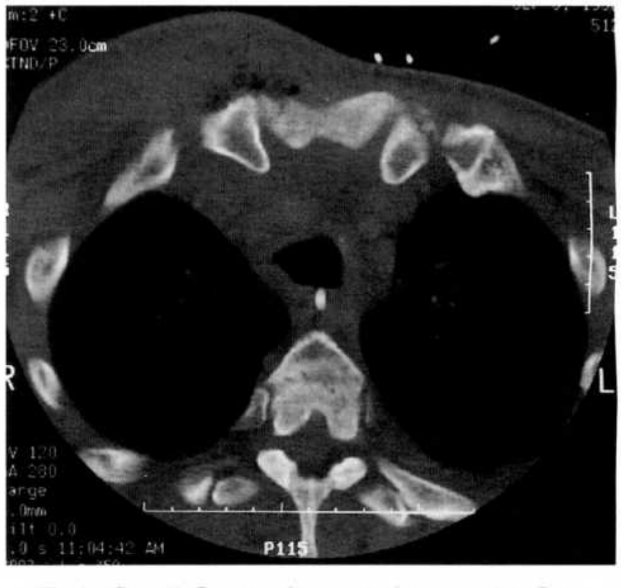

Fig 1. (Case 1) Computed tomography scan taken September 1996, prior to surgery, shows acute osteomyelitis of right clavicle. Subcutaneous air and soft tissue thickening are consistent with abscess formation. Bony cortical erosion of right sternoclavicular joint is present.

From the Department of Otolaryngology–Head and Neck Surgery, University of Colorado Health Sciences Center, Denver, Colorado.

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CORRESPONDENCE — Catherine P. Winslow, MD, Dept of Otolaryngology–Head and Neck Surgery, University of Colorado HSC Box B-210, Denver, CO 80262.

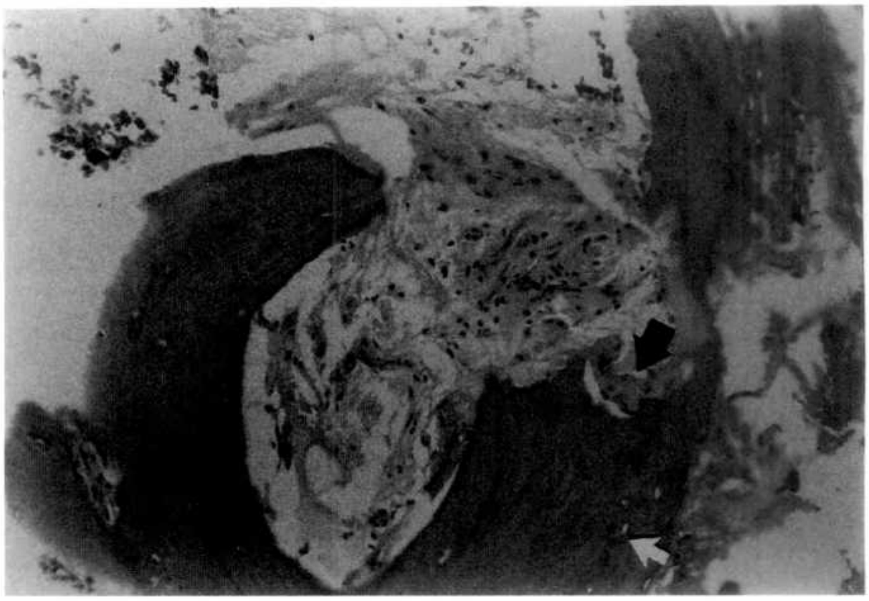

Fig 2. (Case 2) Low-power histologic section of medial clavicle specimen taken November 1996. Bony cortical erosion with remodeling (dark arrow) is accompanied by numerous inflammatory cells and tissue fibrosis. Osteocyte death is evident (note absence of osteocytes in lacunae in area of cortical erosion; light arrow), giving rise to necrotic foci of bone.

Blood cultures grew group A *Streptococcus* and *Pasteurella*. The patient returned to the otolaryngology clinic several weeks later, and the infection had resolved.

Case 2. A 72-year-old man seen at the local Veterans Administration hospital underwent a laryngopharyngectomy and bilateral neck dissection in September 1991 for T2N0M0 squamous cell carcinoma. Postoperative radiotherapy was administered in 1991 for positive margins, and the patient was noted to become hypothyroid following completion. The patient was also noted to have ulcerations over the left clavicle during and following radiotherapy. The patient did well until May 1994, when he presented with pain with left shoulder movement and erythema over the left side of the chest. Needle aspiration of this area grew many group A *Streptococcus* organisms and a few *Staphylococcus aureus* organisms. The patient was taken for debridement, and the medial third of the left clavicle was removed. A pectoralis flap was used to cover the defect. The patient was treated with intravenous, followed by oral, antibiotics. He improved postoperatively and had normal range of motion in the left arm. However, the erythema and tenderness returned after several months, fluctuating in severity. Multiple biopsy specimens of the area were taken, all without evidence of malignancy. This low-grade course of disease continued until November 1995, when the patient was admitted for fever, limited range of left arm movement, erythema, and crepitance over the left side of the chest. A CT scan confirmed osteomyelitis. The patient returned to the operating room for resection of the medial two thirds of the clavicle. Cultures grew *S aureus*, and pathology showed focal marrow fibrosis with new bone formation. The patient did well until return of the osteomyelitis, which was confirmed in November 1996 with CT and magnetic resonance imaging (Figs 2-4). The patient then underwent resection of the manubrium with an omental flap covering the defect. He currently is doing well, without

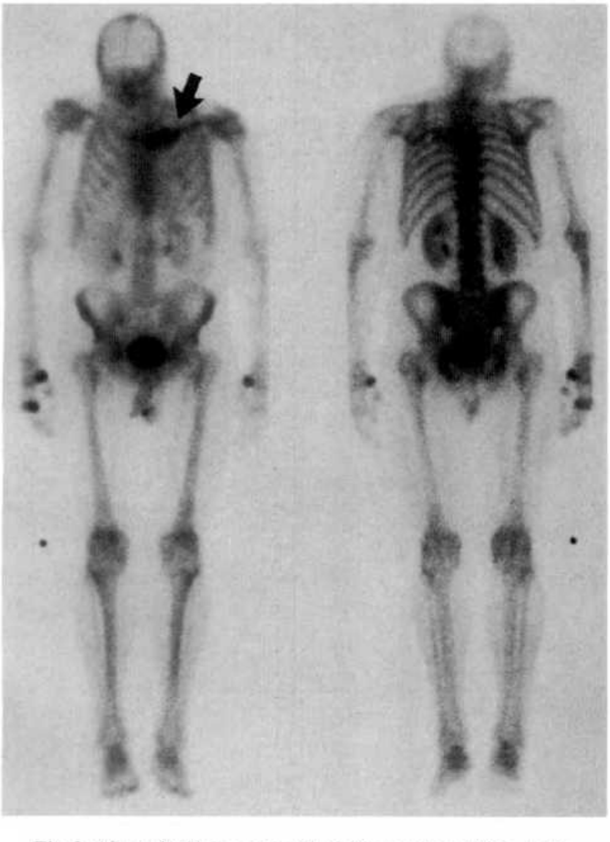

Fig 3. (Case 2) Bone scan taken November 1996; static image with technetium 99 pyrophosphate shows focal area of increased uptake (arrow) quite evident at left medial clavicle and manubrium, consistent with clinical diagnosis of osteomyelitis.

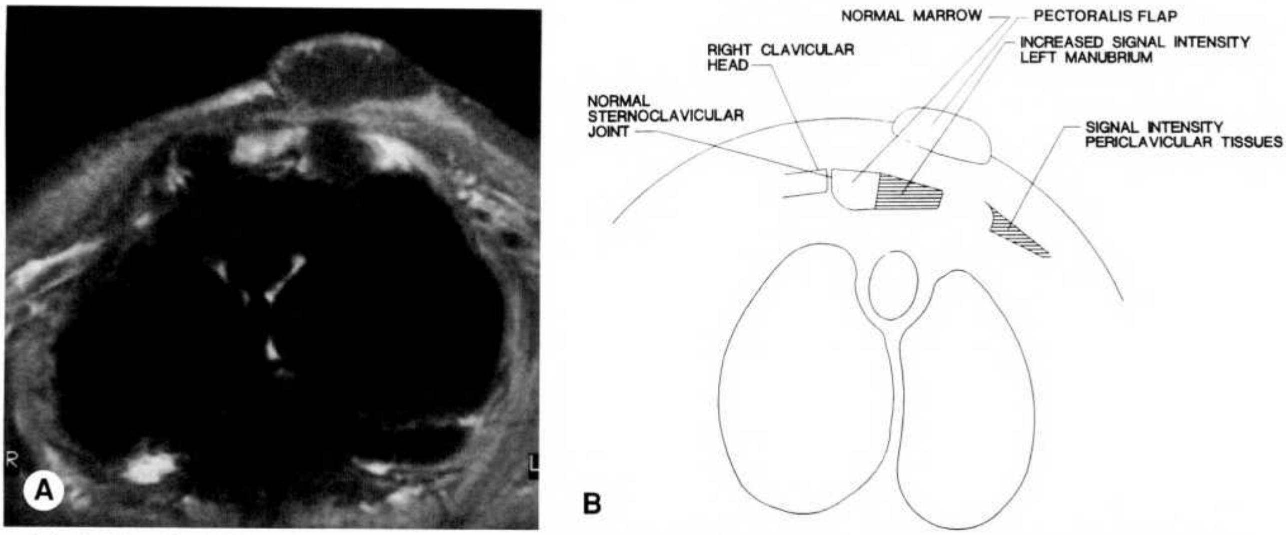
R
L
A
B
NORMAL MARROW
PECTORALIS FLAP
INCREASED SIGNAL INTENSITY LEFT MANUBRIUM
RIGHT CLAVICULAR HEAD
NORMAL STERNOCLAVICULAR JOINT
SIGNAL INTENSITY PERICLAVICULAR TISSUES

Fig 4. (Case 2) A) T2-weighted magnetic resonance image. Pectoralis flap covers area of prior resection. Right clavicular head and manubrium appear normal, with dark signals. Left manubrium is bright, consistent with edema and osteomyelitis. Focal area of bright signal lateral to manubrium may represent inflammation or infection of periclavicular tissues. B) Diagram of these changes.

evidence of infection.

DISCUSSION

Two additional cases of clavicular osteomyelitis were identified on chart review. Due to the relative rarity of this entity, these cases have been included in Table 1. Additional cases of clavicular osteomyelitis following head and neck surgery have been summarized in Table 2.[1-3]

The clavicle is an exceedingly rare site for osteomyelitis.[4] Infection may occur from hematogenous dissemination, spread of contiguous infection, or direct traumatic seeding of bacteria. It has been reported infrequently following central line and Swan-Ganz catheter placement[5,6] and as a result of head and neck surgery. Predisposing factors include prior radiotherapy, loss of periosteal integrity, systemic bacterial infections, and immunosuppression.[7] Diabetes increases the risk of infection due to defective phagocytosis, small vessel disease, and delayed wound healing. Patients with head and neck cancer commonly are malnourished and have vascular disease, often related to years of alcohol and tobacco abuse. Pharyngocutaneous fistula may be a risk factor for clavicular osteomyelitis due to direct seeding of oral flora in a healing wound bed. Patients receiving radiotherapy are predisposed to local vascular compromise. Additionally, a 20% to 30% incidence of hypothyroidism has been noted following radiotherapy alone, which may increase to 70% to 80% following laryngectomy with subsequent radiotherapy.[8] This may be associated with delayed wound healing, and often is only detected with appropriate laboratory surveillance.

The pathogenesis of osteomyelitis is well known. Infection of the bone leads to an inflammatory reaction, which, through increased intraosseus pressure, compromises the blood supply. Purulence dissects the periosteum from the bone, furthering the vascular compromise. Bony necrosis serves as a nidus for further bacterial involvement. Sinus tract formation is not uncommon,[9] due to dissection through the subcutaneous tissue planes. Chronic osteomyelitis has also been described, although there is no clear separation between this and the acute form. Chronic cases present as infections that persistently recur despite treatment. These infections are often associated with a bony sequestrum. Treatment is as for acute infections, ie, aggressive surgical debridement and antibiotics.

The bacteria found in osteomyelitis are *S aureus* in 80% of cases. Other commonly cultured organisms include *Klebsiella* and *Staphylococcus epidermidis*.[4] However, osteomyelitis related to surgery of the head and neck is usually positive for *Streptococcus* species. *Bacteroides* and *Escherichia coli* have also been reported. Gram-negative organisms and anaerobes are frequent offenders. Blood cultures are positive in 50% of patients.

Symptoms occur acutely. These include fever, shoulder swelling, stiffness of the affected arm, and decreased range of motion. A history of prior trauma and/or local surgical procedures may be elicited. Chronic osteomyelitis may present with a history of prior infections and a draining sinus tract or extrusion of necrotic bone.[9]

Diagnosis is based on bacteriologic, radiologic, and pathologic findings. Of paramount concern in

TABLE 1. PATIENTS IDENTIFIED WITH CLAVICULAR OSTEOMYELITIS

	Case 1	*Case 2*	*Case 3*	*Case 4*
Age	60	72	49	51
Sex	M	M	M	M
Prior radiotherapy	Yes	Yes	No	No
Prior surgery	Stomatoplasty	Laryngectomy	Laparoscopic cholecystectomy	Unknown
Cause	Postoperative pharyngocutaneous fistula	Unknown	Unknown	Unknown
Side	Right	Left	Right	Left
Medical problems	Hypothyroidism	Hypothyroidism	Insulin-dependent diabetes mellitus	Hepatitis C, hypothyroidism
Duration	1½ months	2½ years	1 month	3 months
Symptoms	Chronic pain with acute worsening, fever, diarrhea, erythema	Pain with shoulder movement, erythema, crepitus, shortness of breath	Pain, erythema, tenderness, decreased range of motion	Pain, tenderness, decreased range of motion and strength
Diagnosis	CXR suspicious for pneumonia, CT abnormal	CXR normal, CT and MRI abnormal	CXR normal, CT abnormal	CXR normal, CT abnormal
Treatment	Medial claviculectomy	Medial claviculectomy ×2 with pectoral flap, manubrium resection with omental flap	Sternal debridement ×2 with medial claviculectomy and omental flap	Medial claviculectomy with pectoral flap
Culture	Group A *Streptococcus*, *Pasteurella* (blood)	Group A *Streptococcus*, *Staphylococcus aureus* (wound)	*Staphylococcus aureus* (wound)	*Staphylococcus epidermidis*, *Escherichia coli* (wound and blood)
Subclavian catheter use	No	No	No	No

CXR — chest x-ray, CT — computed tomography, MRI — magnetic resonance imaging.

head and neck cancer patients, of course, is recurrence of malignancy. Biopsies as well as cultures should be performed in the area of involvement. Primary clavicular neoplasms are rare; however, when they occur, they are frequently malignant.[10] Sternoclavicular arthritis, osteoradionecrosis, and aseptic necrosis may manifest in a similar clinical fashion, and should be considered in the differential diagnosis.

Radiologic diagnosis may be based on chest X-ray, bone scan, and/or CT scan. Plain films often do not become suspicious until 7 to 10 days following the onset of infection. They may reveal widening of the bony cortex and joint, irregular destruction, a "moth-eaten" appearance, and periosteal new bone formation and thickening.[11-13] A sclerotic or lytic appearance may be observed. Computed tomography may delineate abscess formation and subcutaneous or intraosseous gas formation, but the specificity of this study is poor. Magnetic resonance imaging may be of assistance if the diagnosis is uncertain. It may be as specific as and more sensitive than the 3-phase bone scan, and is useful in distinguishing cellulitis from osteomyelitis.[11] Scintigraphy reveals changes before they are evident on plain films, and thus is important in early diagnosis.[11] However, the local soft tissue infection so commonly seen with these infections can greatly decrease specificity. The 3-phase bone scan helps distinguish soft tissue inflammation from bony involvement, although the false-positive rate is still high. The sensitivity of ^{99}Tc-MDP in combination with plain films approaches 95%. The specificity is improved by adding either a ^{67}Ga-citrate or a ^{111}In-labeled WBC scan.[11] This combination offers increased specificity over the CT scan alone.[14] The positive predictive value of ^{111}In-WBC SPECT is 89%, with a sensitivity of 73% to 100%.[3,12] This study also is useful in detecting abscess formation. However, the specificity in postoperative patients remains imperfect.

Treatment is aimed at the eradication of infection. Intravenous antibiotics should be instituted as soon as the diagnosis is suspected. Therapy should be continued for 4 to 8 weeks. Wide debridement is the mainstay of therapy. Early and aggressive surgical debridement is recommended.[8,13] The potential for complications as severe as mediastinitis, sepsis, and death underscores these principles. Clavicular osteomyelitis often involves the sternoclavicular joint[2,10]; this area should be explored surgically to ensure eradication of infection. Total excision of the clavicle causes no functional disability,[1,13,15] although tem-

TABLE 2. PREVIOUSLY DOCUMENTED CASES OF CLAVICULAR OSTEOMYELITIS FOLLOWING HEAD AND NECK SURGERY

	Baratz et al[1]	*Baratz et al*[1]	*Krespi et al*[2]	*Krespi et al*[2]	*Alessi et al*[3]
Age	68	73	60	57	69
Sex	M	M	M	M	M
Prior radiotherapy	No	Yes	No	Yes	Given between first and second debridements
Prior surgery	Oropharyngeal carcinoma resection	Radical neck dissection	Laryngectomy, radical neck dissection, manubrium and medial third of clavicles, mediastinal node dissection	Laryngectomy	Left radical neck dissection, supraglottic laryngectomy, postoperative pharyngocutaneous fistula
Cause	Cellulitis at tracheotomy site	Infection at tracheotomy site	Medial clavicles disrupted with primary surgery	Purulent tracheitis	Postoperative pharyngocutaneous fistula
Side	Right	Right	Right	Bilateral	Right
Medical problems	Diabetes	Diabetes	Unknown	Unknown	Unknown
Onset after surgery	2½ months	1 month	10 days	2 weeks	7 weeks
Symptoms	Purulent tracheotomy drainage, cellulitis	Erythema, swelling, fever	Fever, erythema, tenderness, edema	Swelling, tenderness, purulent drainage	Pain, decreased range of motion; 8 months later, returned with sinus tract
Diagnosis	CXR normal, CT soft tissue thickening, biopsy	CXR soft tissue density	Technetium 99 scan diffuse enhancement	Unknown	CT abscess, medial clavicle destruction
Treatment	Debridement, total claviculectomy	Debridement, total claviculectomy, manubrium resection	Debridement, medial claviculectomy; pectoral flap closure 1 week later	Exploration, medial claviculectomies, debridement, pectoral flap	Incision and drainage with partial right claviculectomy; 8 months later, required sternoclavicular resection, pectoral flap
Cultures	*Streptococcus* (wound)	Mixed oral flora: *Streptococcus viridans, Enterobacter aerogenes, Bacteroides*	Group F β-hemolytic *Streptococcus* (wound and blood)	*Streptococcus, Bacteroides* from tracheitis; no wound culture reported	*Pseudomonas aeruginosa, Escherichia coli*, group D *Streptococcus* (wound)
Subclavian catheter use	Unknown	Unknown	Unknown	Unknown	Unknown

porary supraclavicular anesthesia has been reported.[15] One patient in our series who underwent medial claviculectomy (case 2) was noted during a subsequent debridement to have bony regeneration of the clavicle. Complete removal also ensures that devascularized bone does not remain as a nidus for recurrent infections; it should be considered in all mediastinal dissections that necessitate clavicular disruption. Flap coverage of the defect may be required and should be anticipated. Krespi et al[2] have advocated utilizing a myocutaneous flap in the primary procedure if clavicular periosteum is disturbed. Additional coverage between the stoma and clavicle will provide needed vascularization and protect the great vessels. Pectoralis major flaps provide necessary bulk, are well vascularized, and have been reported as being successfully utilized in this regard.[8] Hyperbaric oxygen therapy as adjuvant therapy should be considered in treating refractory chronic osteomyelitis.

Prevention is paramount. Sternoclavicular skeletonization should be avoided when performing stomatoplasty or tracheoplasty whenever possible. Subclavian catheterization also should be avoided, when possible, in conjunction with head and neck surgery, as contamination and bacterial seeding from the surgical site may be caused in a clavicle with periosteal compromise due to traumatic attempts at cannulation.

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MIDDLE EAR MECHANICS

The Second International Symposium on Middle Ear Mechanics in Research and Otosurgery will be held October 21-24, 1999, in Boston, Massachusetts. For information, contact Harvard MED-CME, PO Box 825, Boston, MA 02117; telephone (617) 432-1525; web site http://www.med.harvard.edu/conted/.

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IMAGING CASE STUDY OF THE MONTH

LIPOMA OF THE RETROPHARYNGEAL SPACE

ANTONIO ROSELL, MD
MARIA TERESA LLAVERO, MD
GREGORIO GARCIA-ARRANZ, MD, PHD
JUAN MARTINEZ-SAN-MILLAN, MD, PHD
MADRID, SPAIN

INTRODUCTION

Lipomas consist of adipose tissue cells separated by fibrous trabeculae and circumscribed by a delicate fibrous capsule.[1] Most lipomas are well encapsulated, and they recur locally in 5% of cases. Histologically, these tumors are completely benign, but clinically, their locally invasive behavior has been associated with recurrences. Malignant change in lipomas is rare.[2]

About 13% of lipomas occur in the head and neck; most of them are found in the subcutaneous tissues, and a few in the oral cavity, parotid gland, hypopharynx, larynx, nasopharynx, or parapharyngeal space or intracranially.[3] Therefore, lipomas of the deep tissues in the head and neck are rare, and they have seldom been the object of interest in the literature.[4] Many times, their computed tomography (CT) characteristics allow definite diagnosis prior to pathologic evaluation of the surgical specimen.

We report an interesting retropharyngeal lipoma diagnosed by its CT characteristics and its benign clinical course over 19 years.

CASE REPORT

A 56-year-old woman presented to our otolaryngology service for evaluation of an asymptomatic mass in the posterior wall of the oropharynx in 1978. She had first noted a mild sensation of a mass 2 months previously, but had no other deglutitory or respiratory complaints. Her past medical history was unremarkable.

Physical examination revealed, clearly to inspection, a 3 × 5-cm submucosal mass in the posterior wall of the left side of the oropharynx, just lateral to the midline. The overlying mucosa was intact and normal. Palpation revealed a soft, oval, and nonpainful mass. Examination of the ears, nasal cavity, nasopharynx, hypopharynx, larynx, and neck revealed normal findings. A plain lateral neck radiograph was obtained, showing a large oval radiolucent area in the left retropharyngeal space without destruction of bone walls or upper airway compromise. The patient refused to sign a consent for excisional biopsy of the mass, so we decided to perform a clinical and radiologic follow-up periodically. To date, the patient is 75 years old, she has been followed up in the clinic for 19 years, and there has been no additional change according to history or physical examination.

A CT scan with contrast in 1988 (see Figure, A) showed a well-circumscribed, low-attenuation mass consistent with adipose tissue in the left side of the retropharyngeal space, extending from C1 to C4, without invasion of surrounding structures. In 1997, another CT scan showed the same retropharyngeal mass lesion with a Hounsfield unit of −88. No apparent increase in size or change in shape or density was observed (see Figure, B).

Therefore, after 19 years of follow-up, both the typical radiologic findings and the asymptomatic clinical course confirm that the mass is a lipoma of the retropharyngeal space, although we have not obtained a histologic confirmation through biopsy.

DISCUSSION

This reported case of lipoma is unusual in two respects, ie, its principal site of involvement and its clinical course. To our knowledge, this is the first reported case of deep neck lipoma followed up for many years (exactly 19), and it shows that a retropharyngeal lipoma may not change in shape or size, or invade surrounding structures, over many years. On the other hand, lipomatous tumors are uncommon in this area, being of salivary origin. Neurogenic tumors, chemodectomas, metastatic lesions, and lymphadenopathies are more typical lesions presenting as submucosal masses in the oropharynx. So, many

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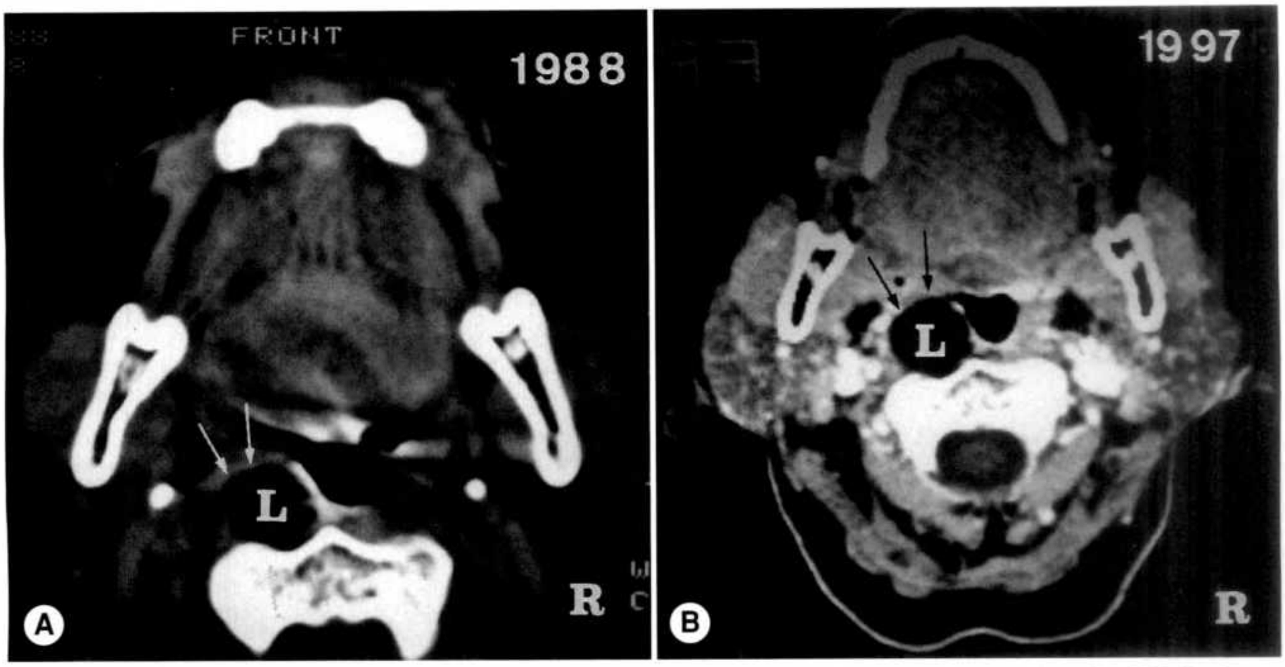
From the Departments of Otolaryngology–Head and Neck Surgery (Rosell, Garcia-Arranz, Llavero) and Radiology (Martinez-San-Millan), Hospital Ramon y Cajal, Madrid, Spain.

CORRESPONDENCE — Antonio Rosell, MD, c/ Bravo Murillo 177, Buzon, 1°-5, Madrid 28020, Spain.



Axial computed tomography scan sections through oral cavity showing location and size of lipoma (L). A) In 1988. Notice that its density (–88 Hounsfield units) is similar to that of fat in superficial soft tissues. Note how soft lipoma conforms to contours of posterior wall of oropharynx (arrows). B) In 1997. No significant changes are observed.

times, establishment of a definite diagnosis may not be possible without pathologic evaluation of a surgical specimen.[2-4]

Unfortunately, it has not been possible to obtain an excisional biopsy of the lesion in our case, because the patient has always refused to sign the consent for it. Indeed, she has never had important complaints. Cytologic examination with fine-needle aspiration biopsy has never been considered. Although current diagnostic imaging techniques (CT and magnetic resonance imaging [MRI]) cannot distinguish a lipoma from a liposarcoma, and the lack of a histologic confirmation may be a handicap in the presented case, we think that the radiologic findings and the clinical course allow us to establish a definite diagnosis of lipoma.[5] Indeed, the characteristic CT appearance of lipoma is that of low attenuation related to fatty tissue (Hounsfield values –50 to –150), such as can be seen in our case. The MRI appearance of lipoma is related to the fat within the tumor: a well-circumscribed mass with a high-intensity signal on T1-weighted images that demonstrates an intermediate signal on T2-weighted sequences. It is not possible to differentiate a lipoma from a liposarcoma on the basis of signal characteristics. A heterogeneous signal may be displayed for a lipoma with fibrosis or for a liposarcoma with hemorrhage and necrosis. So, we did not perform MRI, because it would not have been helpful in the differential diagnosis.

The anatomic features of the retropharyngeal space could explain the clinical course of the case that we present.[6] The retropharyngeal space extends from the base of the skull to the level of the first or second thoracic vertebra, involving the entire length of the neck. It is a virtual space created between the middle layer of the deep cervical fascia that surrounds the pharynx and esophagus anteriorly and the alar division of the deep layer of the deep cervical fascia posteriorly. The retropharyngeal space is separated into two lateral spaces involving an important length of neck by the midline raphe formed where the superior constrictor adheres to the prevertebral division of the deep layer of the deep cervical fascia. The existence of this midline raphe could explain why the lipoma in our case has been well circumscribed for a long period of time. Several other characteristics of the lipoma could also explain its evolution. It is likely well encapsulated and, being soft in consistency, has been able to change shape to accommodate adjacent structures — in this case, the boundaries of the left side of the retropharyngeal space.

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ADVANCED EAR SURGERY

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CLINICOPATHOLOGICAL CONSULTATION
GIANT CELL TUMOR OF THE LARYNX

KENNETH O. DEVANEY, MD
CHICAGO, ILLINOIS

ALFIO FERLITO, MD
UDINE, ITALY

ALESSANDRA RINALDO, MD
PADUA, ITALY

Among the more uncommon tumors that may sometimes be encountered in the laryngeal region is the recently described giant cell tumor of the larynx. This lesion is a true neoplasm, presumably of the fibrohistiocytic series. Histologically, it closely resembles the more familiar true giant cell tumor of long bone. The laryngeal giant cell tumors appear, to date, to be nonmetastasizing lesions; it is possible that they may recur locally if incompletely excised (although this remains to be demonstrated). In view of the rarity of these tumors, a tentative diagnosis of such a neoplasm should always prompt consideration of other (more frequently encountered) differential diagnostic possibilities, including cytologically malignant giant cell–rich tumors such as malignant fibrous histiocytoma and sarcomatoid carcinoma.

HISTORICAL BACKGROUND AND TERMINOLOGY

The giant cell tumor of the larynx is a rather recently described entity, and as a consequence does not usually enter into the clinical differential diagnosis of a laryngeal mass. Rather, it may be expected to occasion some discussion on pathologic examination, at which time it will become apparent that the tumor's appearance differs greatly from that usually encountered in the practice of head and neck pathology.

While experience with these lesions is necessarily limited by their rarity, it seems (at least at this stage in our knowledge) reasonable to draw a parallel between these peculiar tumors of the larynx and the histologically similar giant cell tumors of the long bones. In the skeletal system, true giant cell tumors have a very real potential for recurring locally; in addition, some 5% or so of them lead to the development of (histologically benign) pulmonary metastases; that is, the bony tumors may sometimes behave as "benign metastasizing tumors."[1-4] For the purposes of the discussion below, the laryngeal tumors will be treated as biologically similar locally aggressive neoplasms.

Anatomically, the laryngeal giant cell tumors usually seem to arise in association with the osteocartilaginous supporting structures of the larynx proper; that is, they do not seem to be discrete soft tissue masses. While this is an observation that seems to be a predictable one (in view of the apparent histologic kinship between the laryngeal lesions and the more familiar lesions of long bone), it should be noted that the laryngeal tumors do not actually appear to arise within a bony structure, as do the skeletal tumors. Rather, they typically erode and replace to some extent the (principally cartilaginous) tissues of the affected portion of the larynx. Whether this makes these laryngeal tumors "true" tumors of bone, or instead soft tissue tumors mimicking a skeletal tumor, is speculative.

PATIENT CHARACTERISTICS AND CLINICAL MANIFESTATIONS

In a recent review of the literature, Werner et al[5] identified only 18 cases of giant cell tumor of the larynx. The lesion involves adult males, in their third through sixth decades (average age at diagnosis 40.2 years); female patients have not to date been reported to have developed this tumor (which contrasts with the experience with giant cell tumors of bone, in which female patients outnumber male patients by 2 to 1).[3,6] Presenting problems have included the presence of a slowly growing mass and dysphonia. On physical examination, a mass has usually been palpable; endoscopically, the lesions have proven to be deeply seated, with an intact overlying mucosa.[5-17] Laryngeal giant cell tumors most commonly originate in the thyroid cartilage.[5]

RADIOGRAPHIC FINDINGS

As most giant cell tumors of the larynx are not for the most part mineralized, they appear simply as soft

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From the Department of Pathology, University of Chicago, Chicago, Illinois (Devaney), and the Department of Otolaryngology–Head and Neck Surgery, University of Udine, Udine (Ferlito), and the Department of Otolaryngology, University of Padua, Padua (Rinaldo), Italy.

CORRESPONDENCE — Alfio Ferlito, MD, Dept of Otolaryngology–Head and Neck Surgery, University of Udine, Policlinico Città di Udine, Viale Venezia 410, 33100 Udine, Italy.

tissue densities on plain film radiographs (some peripheral ossification may be appreciated). Computed tomography scanning will reveal a mass with a density intermediate between muscle and fat, which may or may not show some central cystic change.[5,6] While magnetic resonance scanning features have not yet been reported, it is likely that these tumors will prove to be inhomogeneous, with intermediate densities on T1-weighted scans and high to intermediate densities on T2-weighted scans. Reports of scintigraphic findings are likewise lacking, but it is likely that these tumors will not show a striking uptake of tracer.

PATHOLOGIC FINDINGS

Grossly, laryngeal giant cell tumors are soft red-purple lesions, with nodules of gray-tan tissue distributed throughout; a portion of the periphery of the mass may appear to be ossified. While a relatively sharp line of demarcation typically separates the tumors from the adjacent host tissues, a true encapsulation is not present.

The microscopic correlates of the gross findings include abundant hemorrhage (the red-purple coloring and soft consistency), masses of closely packed mononuclear and multinucleated cells (the gray-tan nodules), and foci of peripheral ossification. On closer scrutiny, the cellular zones resolve into two distinct cell types: abundant cytologically benign mononuclear cells, reminiscent of histiocytes, and only slightly less abundant cytologically benign multinucleated giant cells (indistinguishable from the multinucleated osteoclasts normally found within the bone; see Figure). These two cell types are, in turn, set in a richly vascular stroma with some fibrous underpinnings.

Of greatest import in the analysis of this tumor is an assessment of the cytologic features of the component tumor cells; both the multinucleated (osteoclast-like) giant cells and the mononuclear (histiocyte-like) cells are cytologically benign. In fact, the nuclei of these two cell types resemble one another (see Figure, B). While scattered mitotic figures may be encountered here, they are neither atypical nor numerous (that is, they should not number more than 2 to 5 per 10 high-power fields). Hemorrhage, as noted in the initial gross examination of the specimen, is a frequent finding in these tumors; however, frank necrosis of the individual tumor cells themselves is not common.

Immunohistochemically, the fibroblasts, histiocytes, and giant cells express vimentin. The histiocytes and the giant cells are also positive for macrophage markers KP1 and KiM1p. The cytokeratin reaction is negative.[5]

COURSE OF DISEASE AND TREATMENT

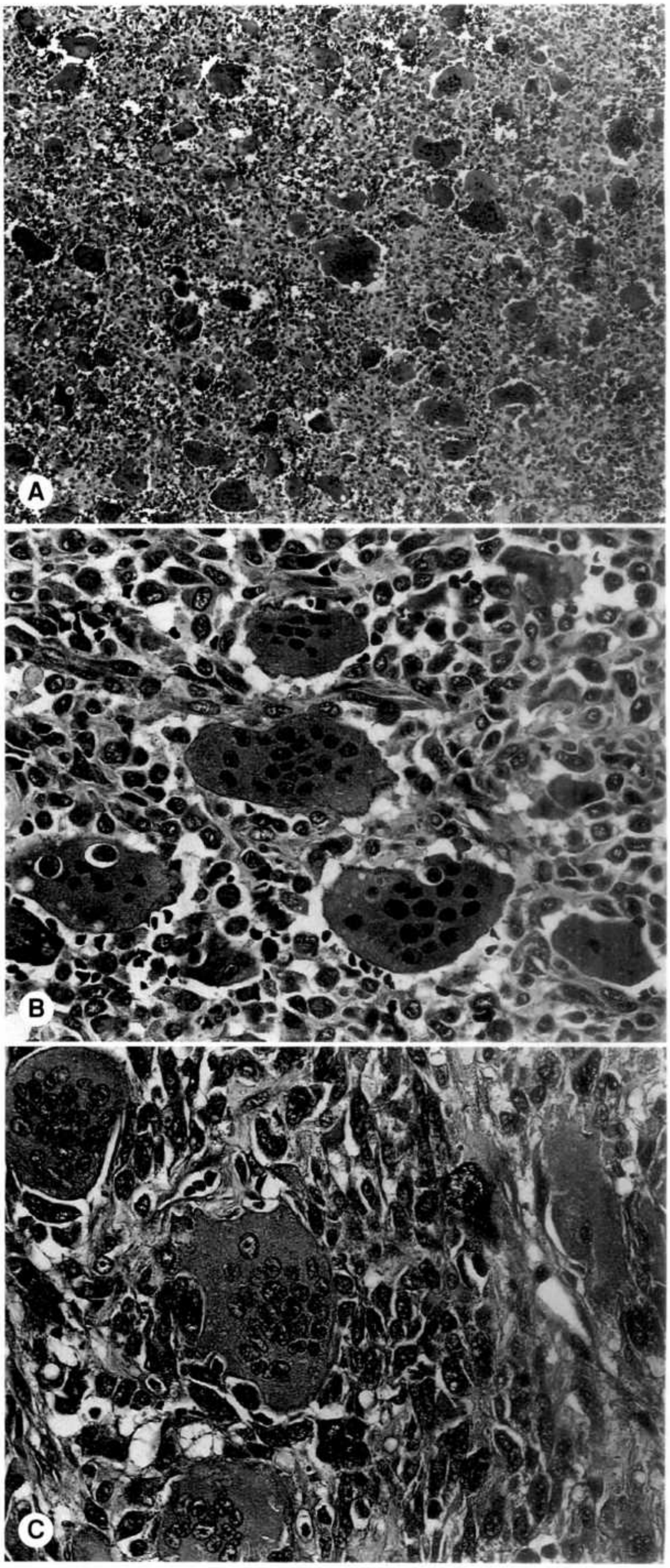
Although older reports suggest that radiotherapy may have been of some use in the control of these tumors, modern therapy usually calls for either a laryngectomy or a hemilaryngectomy, both to achieve local control of the lesion and to obtain sufficient tissue to confirm the pathologic diagnosis. While reports exist of tumors excised with microscopic positive margins that nonetheless failed to recur, it should be a goal of surgical excision to remove the tumor in its entirety if technically possible.[6]

While there are to date no reports of the development of distant deposits derived from histologically benign laryngeal giant cell tumors, it would nevertheless seem prudent to keep in mind the possibility that such may be reported at some future date. This possibility further reinforces the suggestion made above that complete surgical excision should be attempted for definitive treatment of these tumors. Review of the literature demonstrates that the overall prognosis for these lesions is favorable.[5]

DIFFERENTIAL DIAGNOSIS

The first task of the microscopist when constructing a differential diagnosis for a tumor such as this is to distinguish a malignant histologic appearance from a cytologically benign one. Here, two avenues are open for exploration. First, the multinucleated giant cells may be assessed for malignant features. Then, the smaller mononuclear cells may be similarly assessed for evidence of malignant features. In laryngeal giant cell tumors, both cell types are cytologically benign. One other (particularly rare) benign lesion may enter into the histologic differential diagnosis at this point, and that is the aneurysmal bone cyst.[18] These lesions are marked by hemorrhage, as are the giant cell tumors; however, the density of both multinucleated giant cells and mononuclear giant cells is usually overall much less in the aneurysmal bone cysts, and therein lies the histologic key to their distinction.

Tumors that enter into the malignant differential diagnosis here are of two types: those particularly deceptive tumors in which the mononuclear cells are malignant but the multinucleated giant cells are benign, and those tumors in which both cell types — mononucleated and multinucleated — are cytologically malignant. Malignant tumors that are infiltrated by (benign) osteoclast-like giant cells have been recognized in most organs of the body, ranging from the gastrointestinal tract to the urogenital system and



Photomicrographs. A) At scanning power, laryngeal giant cell tumors are extremely cellular lesions (H & E, original ×50). Cellular zones of laryngeal giant cell tumor comprise two cell types: multinucleated giant cells and mononuclear cells. B) Individual nuclei of mononuclear cells, as well as of multinucleated giant cells, of laryngeal giant cell tumor resemble one another, in that they are cytologically benign and of similar size and staining characteristics (H & E, original ×220). C) While this anaplastic thyroid carcinoma (type of poorly differentiated carcinoma) shows superficial resemblance to benign giant cell tumor of larynx, on closer scrutiny it becomes apparent that mononuclear cells are cytologically malignant; while benign multinucleated giant cells are present here, so, too, are cytologically malignant giant cells (H & E, original ×320).

DIFFERENTIAL DIAGNOSIS OF GIANT CELL–RICH LESIONS OF LARYNGEAL REGION

Cytologically benign	
	Giant cell tumor of larynx
	Aneurysmal bone cyst
Cytologically malignant	
	Malignant fibrous histiocytoma (giant cell subtype; primary or metastatic)
	Sarcomatoid carcinoma with osteoclast-like giant cells (primary or metastatic)
	Anaplastic carcinoma of thyroid
	Osteosarcoma (primary or metastatic)

elsewhere to the head and neck region. These tumors are most often carcinomas, but carcinomas of a particular sort: they are often poorly differentiated and so resemble mesenchymal tumors (so-called sarcomatoid or anaplastic carcinomas). This grouping, then, includes primary tumors such as anaplastic carcinoma of the thyroid gland (see Figure, C) and sarcomatoid carcinoma of the larynx, as well as metastatic sarcomatoid carcinomas with osteoclast-like giant cells.

The true sarcomas that may present a similar appearance include (both primary and metastatic) malignant fibrous histiocytomas (giant cell variant) and osteosarcomas. Finally, both the sarcomatoid carcinomas and the true sarcomas may consist of two populations of cells — mononuclear plus multinucleated — which are both cytologically malignant. The differential diagnostic considerations that enter into the study of a laryngeal giant cell tumor are summarized in the Table.

CONCLUSIONS

As a clinical problem, a true giant cell tumor of the larynx is likely to be mistaken for a (much more commonly encountered) carcinoma of the usual sort. Only by virtue of pathologic examination is the true nature of this tumor likely to come to light. The pathologist who is considering such a diagnosis should make as careful an assessment as possible of the cytologic character of the component tumor cells, so as to avoid confusing a giant cell tumor with a deceptively similar malignant tumor such as a sarcomatoid carcinoma or an anaplastic carcinoma of the thyroid gland.

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LETTERS TO THE EDITOR

To the Editor:

We are very concerned about the article recently published by Berkowitz (Neonatal upper airway assessment by awake flexible laryngoscopy. *Ann Otol Rhinol Laryngol* 1998;107:75-80) in which he concludes that the upper airway in the neonate "can be relatively safely and reliably assessed" by awake flexible laryngoscopy (AFL) alone in a variety of clinical settings other than the operating room. We would agree that AFL is a valuable aid in the assessment of neonates who have upper airway symptoms, since this relatively new instrument provides assessment of function, and to some degree structure, of the vocal cords and above. But we strongly disagree that AFL is the *only* procedure required to evaluate the airway in neonates with stridor, dysphonia, and "respiratory distress." In addition to rigid laryngoscopy and bronchoscopy performed under general anesthesia (to more fully evaluate structure) following AFL, pre-endoscopic radiographic evaluation — not addressed by Berkowitz — may aid in the diagnosis. As we have recently cautioned, the diagnosis of one airway problem diagnosed by AFL, such as laryngomalacia, does not preclude the existence of any other lesion(s) of the airway.[1] We also stressed the need to perform rigid endoscopy as the "gold standard" in *all* patients — absent from the Berkowitz study — when assessing a new diagnostic test, ie, sensitivity, specificity, positive and negative predictive values.[1] We also do not advocate performing initial diagnostic endoscopic procedures outside the operating room for neonates who may have a pathologic condition, such as subglottic stenosis, that might require immediate control of the airway; the subglottis cannot be safely and adequately evaluated by AFL, especially following removal of an endotracheal tube, as was the case in 8% of his babies. Also, stridor or dysphonia, or both, may be caused by gastroesophageal reflux disease, which can be further evaluated by other endoscopic procedures, eg, bronchoalveolar lavage, esophagoscopy, and biopsy. Indeed, Berkowitz did diagnose "laryngitis" in 2 neonates, which may have been due to reflux; some of the 28% of the patients in whom Berkowitz found "no abnormality" might have also been found to have reflux as the cause of their symptoms, if a complete endoscopic assessment had been performed.

There is no question that endoscopy under general anesthesia does not assess the functional aspects of the larynx and trachea. Our illustrious predecessors, such as Chevalier Jackson and Paul D. Holinger, were quite capable of diagnosing functional abnormalities (eg, laryngomalacia, vocal cord paralysis, and tracheomalacia), since they performed awake rigid endoscopy in neonates prior to the advent of modern anesthesia and the availability of new instruments like flexible fiberoptic endoscopes. We encourage the use of AFL to assess the neonatal upper airway, but not to the exclusion of our time-tested traditional, safe, reliable, and effective procedures and methods.

CHARLES D. BLUESTONE, MD
3705 Fifth Ave
Pittsburgh, PA 15213-2583
GERALD B. HEALY, MD
ROBIN T. COTTON, MD

REFERENCE

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To the Editor:

I am grateful to Drs Bluestone, Healy, and Cotton for their comments regarding my recent article. These eminent pediatric otolaryngologists share a vast experience in the management of neonatal airway problems, and the concerns they raised must be addressed, and my assertion that "the neonatal upper airway can be relatively safely and reliably assessed by AFL performed by an experienced clinician" must be substantiated.

While my article did not advocate that AFL should be the only investigation employed for the evaluation of upper airway symptoms in neonates, the data reported in the article support the contention that AFL, when combined with careful clinical assessment and appropriate imaging, is the only endoscopic procedure required in the majority of cases. A number of neonates in this series also underwent evaluation for gastroesophageal reflux together with a variety of other imaging procedures, but these details were not included in the article.

Additional endoscopic evaluation under general anesthetic was clinically necessary in only 15 of 110 neonates, and among these, the procedure was performed purely for diagnostic purposes in 7 neonates. The diagnosis was confirmed in 14 of 15 of these cases, with a new diagnosis, that of tracheomalacia, being made in only 1 neonate. This neonate had previously undergone tracheoesophageal repair, and AFL was performed to rule out unilateral vocal cord paralysis that may have occurred as a complication of surgery. Tracheomalacia was clinically suspected at the time and was subsequently confirmed at bronchoscopy.

While definite conclusions cannot be drawn regarding the diagnostic accuracy of AFL in the remaining 95 neonates in whom clinical assessment, imaging, and AFL were thought sufficient to make rigid endoscopy unnecessary, these neonates "generally followed a clinical course consistent with their AFL diagnosis." The clinical significance of any additional airway abnormality that might have been found in this group by rigid endoscopy would therefore be questionable.

In acknowledging the value of AFL in assessing the neonatal airway, Drs Bluestone, Healy, and Cotton limit its use to visualizing structural abnormalities to the level of the vocal cords and above. In my article, the subglottis was adequately visualized in 66 of 101 neonates overall, but where no abnormality was found on AFL — that is, there was no abnormality of the vocal cords, supraglottis, or pharynx to obscure the view — the subglottis was adequately visualized at AFL in 25 of 26 neonates.

Drs Bluestone, Healy, and Cotton do not advocate performing AFL outside the operating room if significant airway disease is present. Clearly, every referral for airway assessment is based to some degree on a presumption that a significant pathologic condition exists, which would therefore suggest that Drs Bluestone, Healy, and Cotton recommend that all AFLs should be performed in the operating room. While my article emphasizes the importance of adequate monitoring and resuscitation facilities being available at the time of AFL, none of the neonates were assessed in the operating room, and no major complications occurred. Evaluation of the neonatal airway by AFL at the bedside rather than the operating room has major advantages. It allows expeditious assessment and immediate diagnosis to be made, and facilitates planning of further investigations and appropriate therapeutic intervention. Further, the availability of a quick, simple, relatively safe, and inexpensive technique for airway assessment provides an incentive for our neonatal colleagues to refer patients for otolaryngology evaluation, when they otherwise might not have felt that referral for more detailed evaluation was warranted. Thus, AFL encourages early referral and discourages the neonatologist from overlooking potentially significant airway abnormalities.

Rigid endoscopy has, and will continue to have, a paramount role in the assessment of the neonatal airway. The routine use of rigid endoscopy in all neonates with upper airway symptoms should, however, be questioned.

Robert G. Berkowitz, FRACS
Royal Children's Hospital
Melbourne, Australia

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CORRECTION

An error appears in the article by Drs Liancai Mu and Ira Sanders, "Neuromuscular Organization of the Human Upper Esophageal Sphincter" (*Ann Otol Rhinol Laryngol* 1998;107:370-7). Figure 1B is incorrect (see page 372). We reproduce below the corrected version of the illustration. We apologize for the mistake.

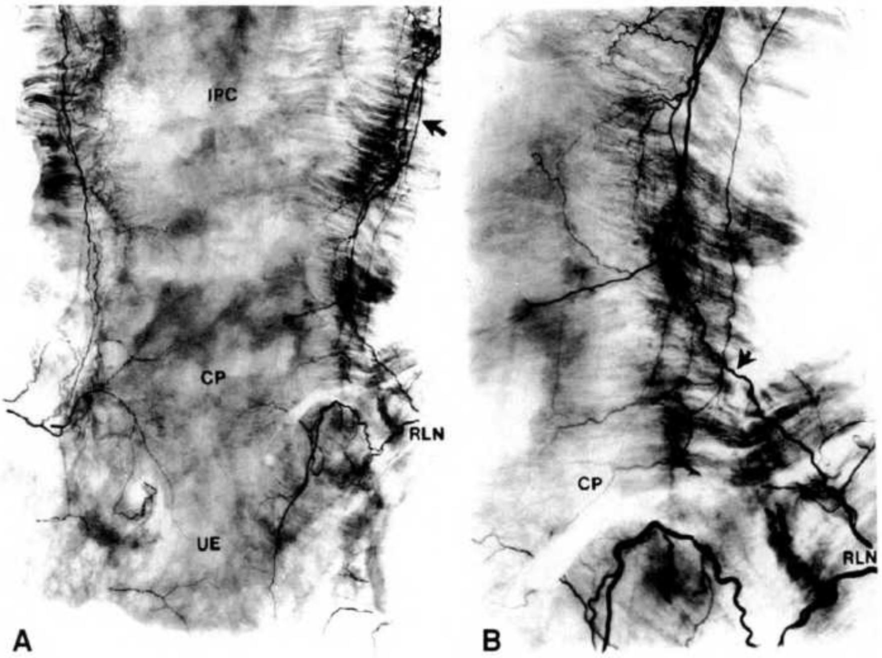

Fig 1. A) Human upper esophageal sphincter (UES) after Sihler's stain (posterior view), showing innervation patterns of each component of UES. Arrow — pharyngeal plexus, IPC — inferior pharyngeal constrictor muscle, CP — cricopharyngeal muscle, UE — upper esophageal muscle, RLN — recurrent laryngeal nerve. B) Higher-power view of nerve supply of CP. Arrow indicates neural connection between RLN and pharyngeal plexus (nerve branches descending from top).

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LATERAL LARYNGOTOMY FOR THE REMOVAL OF TEFLON GRANULOMA

JAMES L. NETTERVILLE, MD
JOHN R. COLEMAN, JR, MD
SUSAN CHANG, MD
CHERYL L. RAINEY, PHD
LOU REINISCH, PHD
ROBERT H. OSSOFF, MD
NASHVILLE, TENNESSEE

Teflon injection has been used for vocal fold medialization following paralysis. Recently, numerous articles have discussed the complications of Teflon injection, including overinjection, airway obstruction, Teflon granuloma, and an abnormal mass effect creating a decreased vibratory character of the true vocal fold. Multiple techniques for Teflon removal have been described. This report details our experience with complete Teflon granuloma removal via a lateral laryngotomy under local anesthesia. Microscopic dissection of the entire granuloma and the paraglottic space was accomplished in all patients. Due to extensive destruction caused by the granuloma, the vocal ligament was resected in 3 patients; it was partially resected and reanastomosed in 1 case, and spared in 6 patients. Laryngeal reconstruction was accomplished with an inferiorly based sternohyoid muscle rotation flap and arytenoid adduction. Effortful speech secondary to pressed vocal quality resolved in all patients. Near-normal to normal vocal quality was obtained in 4 patients, with the average "voice desirability" improving 60% and the effective glottic width increasing 29%. Factors that contributed to a successful outcome included noninvolvement of the vocal ligament and sparing of the mucosal cover.

KEY WORDS — granuloma, laryngotomy, Teflon.

INTRODUCTION

The management of unilateral vocal fold paralysis has been an evolving process for many years. Since its introduction by Arnold[1] in 1962, the injection of polytetrafluoroethylene (Teflon) has gained widespread use. Proponents of Teflon cite the ease of injection, the inexpensive cost, and the relatively few complications.[2] Reported complications are varied, with the most significant being airway obstruction, overinjection, and Teflon granuloma.

Recently, there has been increased attention focused on the long-term effects of the intrafold injection of Teflon. The inflammatory response to Teflon, which occurs in every case of injected Teflon, has been well characterized.[3,4] In some patients, the inflammatory response does not expand enough to become clinically evident. However, as more attention has been focused on long-term outcomes, it is apparent that there is a significant rate of clinically evident granuloma formation in many patients. Hoarseness and vocal strain slowly increase as the stiffness of the vocal fold increases. The pressed or strained voice quality occurs as the granuloma extends across the midline. Varvares et al[5] describe two possible outcomes for Teflon injection: patients who show no improvement and patients who show some improvement initially and subsequently develop progressive dysphonia. They postulate that the progressive dysphonia results from operator error with the position or volume of injected Teflon, the inflammatory response, or a combination of both.[5]

A consistently effective treatment to address the vocal aberrations seen in patients with clinically significant granuloma has not been developed. Treatment efforts have concentrated on either partial or total excision of the granuloma. Different authors have proposed a variety of techniques for Teflon removal. Rubin[6] first commented on Teflon removal and proposed removal with preservation of the medial margin of the vocal fold. Dedo[7] further modified this approach by accessing the Teflon endoscopically through a superior fold incision. Kasperbauer et al[8] preferred removal of Teflon and the granuloma matrix through a lateral cordotomy. Two reports, one by Russell et al[9] and one by Pohris and Kleinsasser,[10] proposed midline thyrotomy with removal of the Teflon and surrounding tissue. Reconstruction following this near-hemilaryngectomy was accomplished with pedicled muscle flaps. Ossoff et al[11] endoscopically used the carbon dioxide laser and a superior vocal fold microflap incision for subtotal excision of the granuloma. In some patients, multiple procedures were required to obtain adequate results. Dedo[12] has written that the potassium titanyl phosphate laser is the laser of choice for removal, while Bryant et al[13] have found the free-electron la-

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From the Department of Otolaryngology–Head and Neck Surgery, Vanderbilt University Medical Center, Nashville, Tennessee.

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CORRESPONDENCE — James L. Netterville, MD, Dept of Otolaryngology–Head and Neck Surgery, S-2100 Medical Center North, Vanderbilt University Medical Center, Nashville, TN 37232-2559.

ser tuned between 8.25 and 8.75 μm to be particularly effective at Teflon ablation in an animal model. A central theme in the recent literature on Teflon granuloma removal is the difficulty of removal, with the frequent need for multiple procedures to produce the desired effect. Even when a reasonable voice improvement is obtained with partial excision, slowly progressive dysphonia will develop with further growth of the residual granuloma.

In this paper, we describe a new method of Teflon removal and provide quantitative and qualitative analysis of our results. We propose the removal of Teflon granuloma through a lateral laryngotomy under local anesthesia with flexible nasopharyngoscope guidance. Dissection of the paraglottic space allows for removal of the entire granuloma. Microscopic surgical technique is required to ascertain the plane of dissection between the granuloma and the vocal ligament and overlying mucosa. If the vocal ligament and a small amount of overlying lamina propria can be saved, there is excellent potential for significant voice improvement.

MATERIALS AND METHODS

Experimental Design. Patients eligible for this study underwent Teflon injection at some point in the past for a unilateral vocal fold paralysis and developed signs or symptoms consistent with granuloma formation. Prior to the surgical procedure, the patients were evaluated with a history and a physical examination including videostroboscopy, complete voice analysis, laryngeal function tests, and a computed tomography scan of the larynx to assess the extent of granuloma formation. Patients were followed closely after Teflon excision, with repeat voice analysis at 3, 6, and 12 months following surgery. Patients were seen annually after the initial postoperative year. The methods of voice analysis are detailed below.

Voice Evaluation. At the time of initial presentation to the clinic, all patients underwent a battery of testing procedures to document and evaluate the voice and laryngeal status. The testing protocol included a voice sample and videostroboscopy. The patient was asked to read the phonetically balanced Rainbow Passage and sustain the vowel "ah" at a comfortable pitch and loudness level. All voice samples were recorded on videotape with a Panasonic SVHS recorder (model AG-7350), a Toshiba microchip camera, and a Sony electret microphone (model ECM-55B) with a mouth-to-microphone distance of approximately 10 cm. To examine the laryngeal anatomy and the vibratory characteristics of the vocal folds during phonation, videostroboscopy was performed on each patient with a Machida 70° rigid endoscope and a Bruel and Kjaer stroboscope (type 4914). Of particular interest was the glottic configuration created by the Teflon granuloma and the effect of the granuloma on the mucosal wave of the vocal fold.

Voice Analysis. In order to determine perceptual and acoustic differences in preoperative and postoperative voices, the sustained "ah" and 10 seconds of the videotape reading sample were placed onto an optical disk. These voice samples were then copied onto digital audiotape in a randomized order, such that the evaluators would not know whether a sample was obtained preoperatively or postoperatively. Five speech and language pathologists, all specializing in the diagnosis and treatment of voice disorders, made independent evaluations of the voice samples using a nominal scale to assign each patient a Vanderbilt Voice Score. The trained listeners were very familiar with the rating scale, commonly used to evaluate the perceptual results of phonosurgical procedures in our clinic.[14] The parameter rated was "voice desirability," an estimate of how functional the voice was for communication. Disorders of resonance or articulation were disregarded. The scores ranged from 0 to 50, with 40 to 50 being a near-normal to normal score, 30 to 40 representing mild breathiness and/or raspiness but a reasonably functional voice, 20 to 30 connoting moderate breathiness and/or raspiness with moderate communicative impairment, 10 to 20 representing a very breathy voice with severe communicative handicap, and 0 to 10 being a profound voice disorder. The five scores assigned to each preoperative and postoperative sample were then averaged to obtain an overall Vanderbilt Voice Score.

Acoustic analysis of preoperative and postoperative sustained "ah" samples was carried out with the Soundscope program (GW Instruments) for the Macintosh computer. This program used a 16-bit analog-to-digital converter, sampling at 44.1 kHz, to digitize an audio waveform for measurement of jitter and shimmer, which are indices of irregularity or perturbation in the acoustic signal and are thought to parallel a perception of abnormal voice quality.

Effective Glottic Width Analysis. In order to calculate the effective glottic width, we used the videostroboscopic examinations. The Panasonic SVHS recorder allows for frame-by-frame advancement. Using the strobe to isolate the glottic cycle, we were able to capture images of the larynx at maximum opening and closure. These images were imported into a Macintosh computer with Screen Play 1.0. They were then converted to a PIC file in Adobe

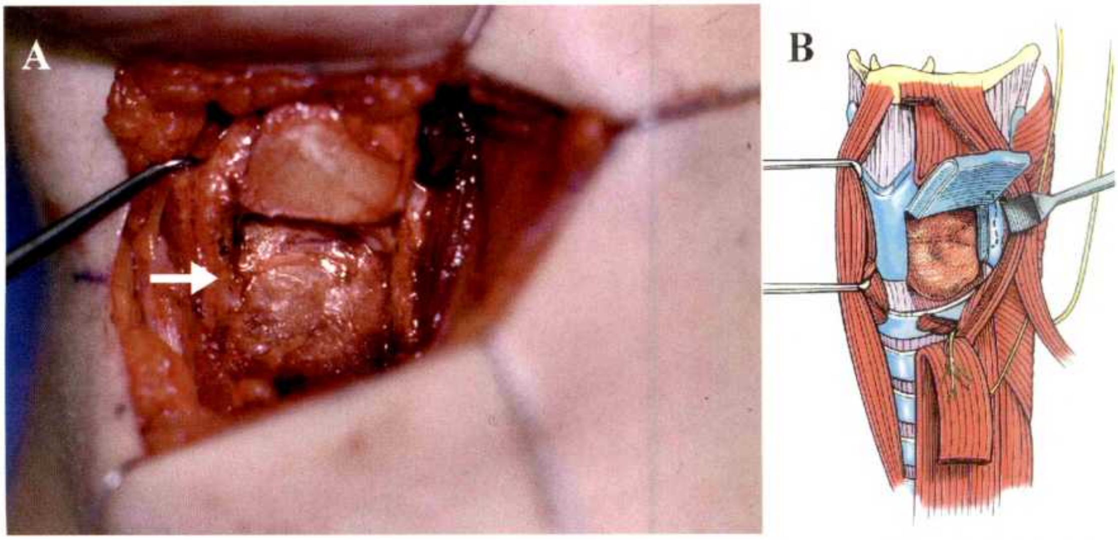

Fig 1. Left-sided approach to paraglottic Teflon granuloma. A) Thyroid cartilage trapdoor has been elevated to show large granuloma. Arrow — level of vocal ligament. B) Corresponding schema. Note that neurovascular complex to muscle flap has been preserved.

Photoshop 2.0.1. Analysis was then performed with NIH Image 1.52. The freeform tool in Image 1.52 was used to outline the glottic area at maximum abduction; the line tool was employed to measure the distance from the anterior commissure to the posterior commissure. The distance from the anterior commissure to the posterior commissure is assumed to remain constant during an examination. By taking a ratio of area divided by height, we are able to estimate the effective glottic width and control for different magnifications seen during an examination due to different angulations of the 70° scope. Three effective glottic widths were obtained from each preoperative and postoperative stroboscopic examination. Each glottic width was calculated from a different glottic cycle.

Surgical Technique. Initially, the surgical procedures were performed under general anesthesia with a tracheotomy placed to secure the airway in the postoperative period in the event of swelling. The tracheotomy was also necessary to remove the endotracheal tube from within the larynx during surgery to allow for arytenoid adduction. Because very little swelling in the postoperative period was noted and patients were decannulated early, we elected to perform the subsequent procedures under local anesthesia with sedation and transnasal fiberoptic monitoring of the larynx, as we have previously described for medialization laryngoplasty.[15] This provides direct visualization of the larynx and immediate assessment of the patient's voice.

The patient is taken to the operating room and placed on the table in the supine position. The nose is anesthetized with 4% cocaine, and a nasopharyngoscope is placed through the nose and suspended over the patient such that the tip of the nasopharyngoscope is above the glottic surface. An incision on the involved side is planned such that it is hidden in a neck crease near the cricothyroid membrane. A combination of 1% lidocaine hydrochloride and 0.5% bupivacaine hydrochloride, both with appropriate concentrations of epinephrine, is used to obtain local anesthesia. The injections are at the incision site and spread superiorly to the level of the hyoid and inferiorly to the lower border of the cricoid. The patient is monitored closely by the anesthesia staff and given intravenous antibiotics, steroids, and sedation as necessary. The sedation is titrated such that the patient can actively participate in the reconstructive phase of the operation.

After the cutaneous incision and the subplatysma flaps are completed, special attention is given to the elevation of the ipsilateral sternohyoid muscle, which will be used for the paraglottic reconstruction. The medial border of the sternohyoid muscle is elevated off the sternothyroid muscle, with identification of the neurovascular pedicle entering the medial surface of the partially mobilized muscle just inferior to the lower border of the thyroid cartilage. The sternohyoid muscle is taken down 2 cm from its insertion on the medial hyoid and retracted laterally, with care to prevent injury to the neurovascular bundle until the reconstructive phase of the procedure.

We have used two different approaches to the paraglottic space. In an effort to visualize the entire paraglottic space, a superiorly based "trapdoor" of thyroid cartilage is outlined (Fig 1). In this technique, the thyrohyoid musculature is left in place; parallel

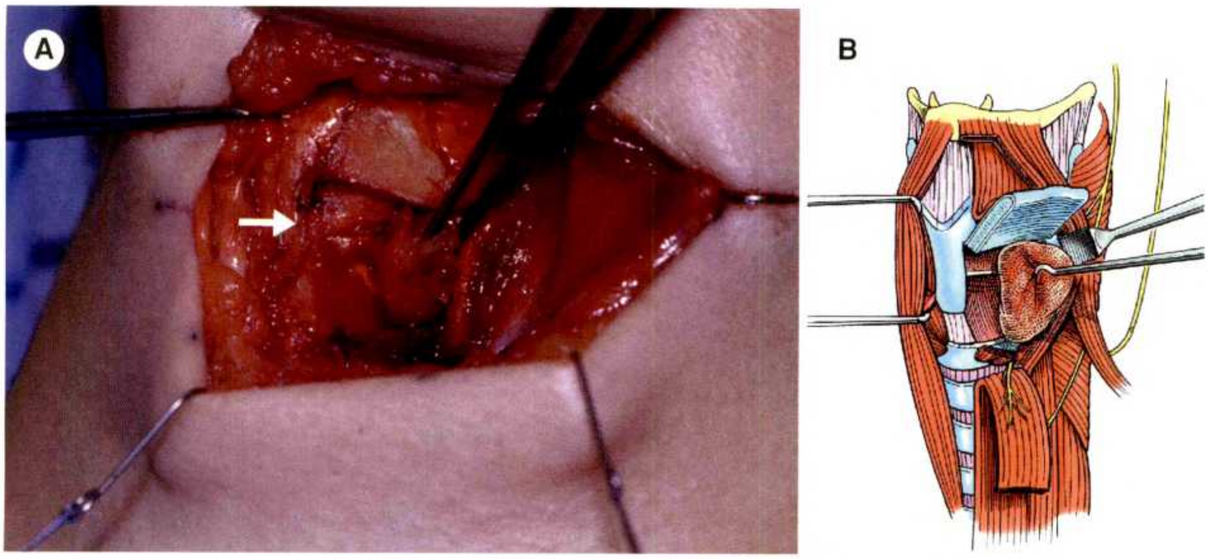

Fig 2. Dissection of granuloma. A) Granuloma is dissected off vocal ligament with attempt to preserve arytenoid and laryngeal mucosa. Complete dissection of paraglottic space and complete removal of granuloma are hallmarks of procedure. Arrow — level of vocal ligament. B) Corresponding schema.

cuts are made in the cartilage at the inferior border to create a "trapdoor" that is superiorly based. The "trapdoor" can be elevated with the thyrohyoid muscle and perichondrium up off the paraglottic space with gentle lateral retraction on the inferior border of the isolated segment of cartilage. After reaching a level superior to the granuloma, the inner perichondrium is divided along the line of the proposed cartilage fracture. After division of the inner perichondrium, the "trapdoor" will fracture pedicled on the outer perichondrium and allow an unencumbered access to the paraglottic space. A microscope is used to better visualize the granuloma and its boundaries. Dissection starts at the anterior edge of the granuloma in the subglottic space (Fig 2). If the vocal ligament is not evident as it is approached from below, the dissection should then proceed from the posterior aspect, peeling the granuloma off the face of the arytenoid until the vocal process and the vocal ligament can be identified. If it is still difficult to identify the ligament, the ventricle is opened and direct visualization of the vocal fold directs the dissection. The major goal of the dissection is the preservation of the vocal ligament and its overlying lamina propria. All uninvolved mucosa is saved. Frequently, mucosa of the ventricle and/or the subglottis is resected due to granuloma involvement (Fig 3).

If the patient has previously undergone a medialization laryngoplasty or if the granuloma is small, access is gained to the paraglottic space through a window in the lateral ala of the thyroid cartilage instead of the "trapdoor." This window is similar in location to, although larger than, the standard window for medialization laryngoplasty.

After complete removal of the granuloma, two dis-

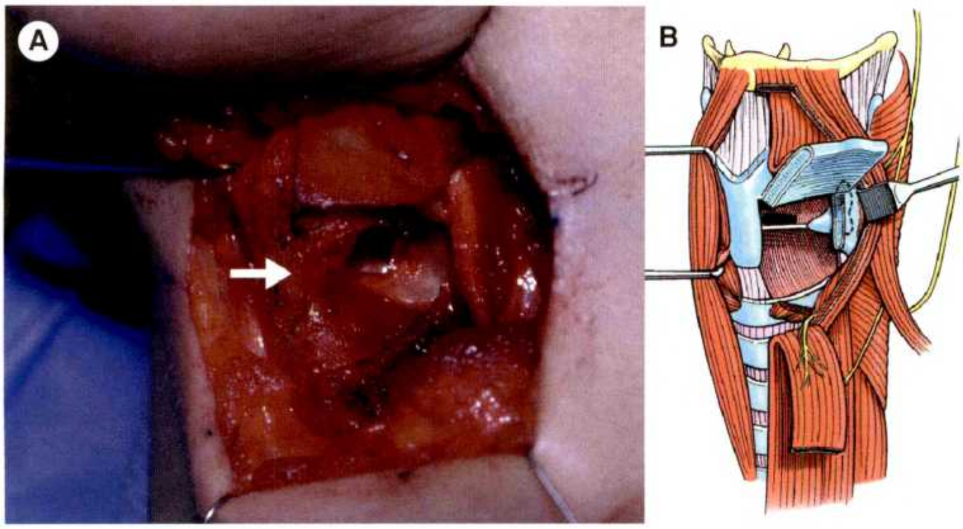

Fig 3. Resulting defect from complete granuloma resection. A) Defect in ventricle has been created during dissection, but vocal ligament and arytenoid have been preserved. Arrow — level of vocal ligament. B) Corresponding schema.

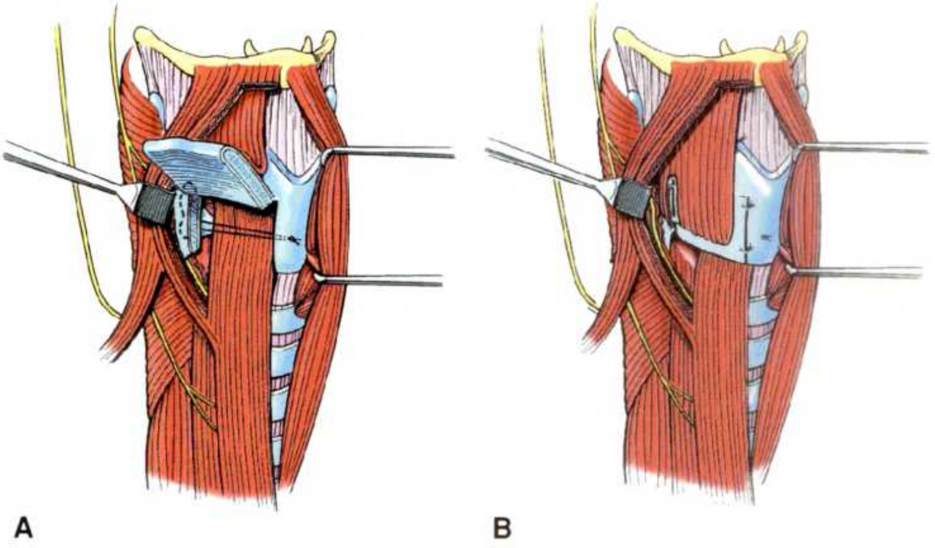

Fig 4. Reconstruction of paraglottic space with sternohyoid muscle flap. A) Muscle flap has been rotated into position under arytenoid adduction stitch. It can be sutured with Prolene suture to superior aspect of thyroid cartilage. B) Trapdoor flap is returned to its native position and held in place by Prolene suture.

tinct arytenoid adduction sutures are placed through the muscular process, which can often be accessed through the enlarged dissection window or "trapdoor." If the access is not sufficient, then the muscular process is approached in the standard fashion around the posterior border of the thyroid cartilage.

The mobilized sternohyoid muscle flap is rotated into the defect (Fig 4A). If the entire vocal ligament and mucosal blanket has been removed, then the superior edge of the muscle flap is pulled medial to the top half of the thyroid cartilage and attached superiorly with sutures ascending out through the thyrohyoid membrane. If the vocal ligament and subglottic mucosa are intact, then the leading edge of the muscle flap is rolled medially to fill the void in the vocal fold, with the more proximal lateral portion of the muscle suspended from the superior edge of the window.

One set of the arytenoid adduction sutures passing lateral to the muscle flap is used to test for voice improvement. It is passed through a drill hole in the anterior thyroid cartilage and tied with the appropriate tension. The second set of arytenoid adduction sutures is placed along the path of the first, but not tied. It is used if revision medialization laryngoplasty is needed. If the mucosa is intact, the field is still sterile, and a silicone implant can be placed to further medialize the muscle flap. If the ventricle or subglottic space is opened, we avoid the silicone implant, as chronic infection can lead to implant extrusion.

The "trapdoor" is brought down into position and sutured with Prolene sutures back into position (Fig 4B). If closure of the "trapdoor" produces a strangulation effect on the muscle flap, the door can be partially closed, or partial resection of the upper lateral border of the cricoid cartilage may further open the passage.

RESULTS

Of the patients presenting to the senior author (J.L.N.) with Teflon granuloma formation since 1993, 12 patients agreed to undergo lateral laryngotomy, total granuloma excision, sternohyoid reconstruction with arytenoid adduction, and medialization laryngoplasty as needed for reconstruction. All patients presented with complaints of a pressed, dysphonic voice with rapid vocal fatigue. Although doing well, 3 patients were excluded from data analysis due to follow-up of less than 6 months after Teflon resection. Table 1 characterizes this patient population and contains a summary of patient demographics and time courses. At the time of operation, all patients were found to have histologically proven Teflon granuloma.

We have also treated 2 patients who had silicone medialization with excessively large implants creating a pressure necrosis on surrounding vocal fold structures. Following removal of these large implants, an identical reconstruction was required to recreate the paraglottic space. Although the cause was different, these 2 patients presented with a similar clinical picture and dysfunctional voice and required the same operations to restore voice and reduce airway resistance. The demographic data of these 2 patients (Nos. 9 and 10) is included in Table 1.

Unfortunately, 1 patient (No. 9) had her stroboscopic examination done before her referral to our center, and the data could not be used for our analysis. In all cases, the postoperative sample was the videostroboscopic examination obtained after the last

TABLE 1. PATIENT CHARACTERISTICS

Case	*Sex*	*Age*	*Cause of Vocal Fold Paralysis*	*Paralyzed Side*	*Teflon Injection Date*	*Teflon Excision Date*	*Time to Teflon Excision*
1	M	32	Motor vehicle accident, prolonged intubation	L	1989	5/11/93	4 y
2	F	63	Thyroidectomy	R	1993	3/25/94	4 mo
3	M	50	Trauma	R	1976	3/30/94	18 y
4	M	32	Motor vehicle accident, prolonged intubation	L	1990	9/16/94	4 y
5	F	46	Subtotal thyroidectomy	L	1993	8/8/95	2 y
6	F	47	Glomus jugulare resection	L	1982	10/31/95	13 y
7	F	45	Congenital	L	1975	11/14/95	20 y
8	M	38	Craniotomy for carotid body tumor	L	1983	11/28/95	12 y
9*	F	47	Schwannoma resection	L	1995	1/19/96	6 mo
10*	M	67	Craniotomy for intracerebral aneurysm	L	1993	4/26/96	3 y
11	M	61	Patent ductus arteriosus repair	L	1980	5/22/96	16 y

*Patients with excessively large silicone implants.

voice-related surgery.

Eleven patients then were successfully treated with complete excision of their Teflon granuloma or removal of their excessively large silicone implant. All patients were reconstructed with a sternohyoid muscle rotation flap (Table 2). In all patients, the pressed speech created by the stiff vocal fold extending across the midline was relieved. In the first 4 patients, tracheotomy was performed at the time of the procedure for airway control. The last 5 granuloma patients, as well as the 2 patients with excessive implants, underwent the procedure under local anesthesia without the need for tracheotomy.

The granuloma involved and required the resection of the vocal ligament and the overlying lamina propria in 3 patients. One patient required partial resection of the vocal ligament and reanastomosis secondary to limited involvement of the ligament in the posterior third of the cord. In the final 5 patients with granuloma, the vocal ligament and the overlying lamina propria were completely saved. Mucosa of the ventricle and/or the subglottic space was partially resected in 2 of these 5 patients. Seven patients underwent arytenoid adduction at the time of the procedure in order to close a posterior glottic gap to allow for improved phonation.

Eight patients required at least 1 follow-up surgical procedure (Table 3). Two patients underwent direct microlaryngoscopy with removal of either a ventricular polyp or granulation tissue. Five patients had a planned second-stage medialization laryngoplasty with silicone. Three patients had augmentation of their reconstruction with either fat or Gelfoam. Finally, 1 patient had a palatal adhesion procedure to reduce nasal incompetence. In sum, 16 postoperative procedures were performed; 12 of these procedures were directly intended to further augment our initial reconstruction.

There were no significant postoperative complications, including no cases of hemorrhage or airway obstruction in the early postoperative period. One patient developed extensive subcutaneous emphysema that resolved over a 3-day period.

Vanderbilt Voice Score Analysis. The results of our

TABLE 2. OPERATIVE DETAILS

Case	*Paralyzed Side*	*Arytenoid Adduction*	*Vocal Ligament*	*Sternohyoid Muscle Reconstruction*	*Tracheotomy*
1	L	No	Resected	Yes	Yes
2	R	Yes	Preserved	Yes	Yes
3	R	No	Resected	Yes	Yes
4	L	Yes	Resected	Yes	Yes
5	L	Yes	Preserved	Yes	No
6	L	Yes	Preserved	Yes	No
7	L	Yes	Resected and reanastomosed	Yes	No
8	L	Yes	Preserved	Yes	No
9	L	Yes	Preserved	Yes	No
10	L	No	Preserved	Yes	No
11	L	No	Preserved	Yes	No

TABLE 3. ADDITIONAL PROCEDURES FOLLOWING INITIAL RECONSTRUCTION

Case	*Fat Injection*	*Fat Injection Date*	*Medialization Laryngoplasty*	*Medialization Laryngoplasty Date*	*Gelfoam*	*Gelfoam Date*	*Additional Procedures*	*Additional Procedure Date*
1	Yes	11/8/94	No		Yes	4/11/94	Laryngoplasty for L ventricle resection	11/23/94
2	No		No		No		Direct microlaryngoscopy for R polyp excision	6/24/94
3	Yes Repeat	1/5/95 7/5/95	No		Yes	5/16/94	Direct microlaryngoscopy for R granulation excision ×2	5/16/94 7/12/94
4	Yes Repeat	12/5/94 5/5/94	Yes	10/20/96	No		None	
5	No		No		No		L palatal adhesion	3/19/96
6	No		Yes	8/16/96	No		None	
7	No		Yes	3/22/96	No		None	
8	No		Yes	11/28/95	No		None	
9	No		Yes	8/16/96	No		None	
10	No		No		No		None	
11	No		No		No		None	

survey of five Vanderbilt Voice Center speech pathologists are displayed in Fig 5. Four patients were described as having perceptually near-normal to normal voices postoperatively; 4 patients were felt to communicate well, but to have mild breathiness or raspiness; 2 patients were characterized as having noticeable impairment with moderate breathiness; and 1 patient was very breathy, with communicative dysfunction.

When we compare preoperative to postoperative voice results, we find that on average, for the 10 patients for whom we recorded preoperative and postoperative values, their voice scores increased by 60% (p = .05). Moreover, if we subdivide our small population into those with intact vocal ligaments and those who had their vocal ligaments removed or reconstructed at the time of surgery, we see a dramatic difference between these groups. Those patients who had an intact vocal ligament show a 102% increase in voice score (p = .02), while those with vocal ligament damage or removal are essentially unchanged postoperatively, showing a 4% decrease in voice score (p = .89).

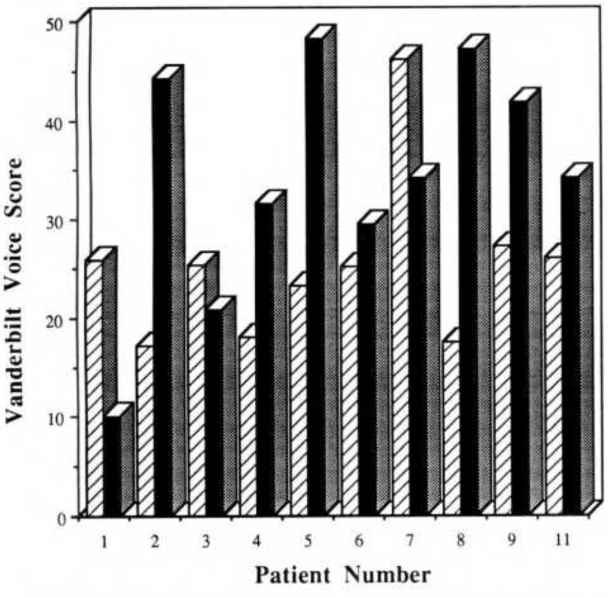

Fig 5. Graph showing preoperative (hatched bars) versus postoperative (solid bars) Vanderbilt Voice Score.

Acoustic Analysis. An interesting trend emerged during the acoustic analysis of voice. When we divide our sample group into those who have vocal ligament preservation and those who have vocal ligament sacrifice or reconstruction, we see a marked difference. In the vocal ligament preservation group, jitter decreased 44% (p = .006), while those with ligament removal or reconstruction showed an increase in jitter of 430% (p = .037). Shimmer values were not statistically different between the two groups.

Effective Glottic Width Analysis. From our analysis of preoperative and postoperative videostroboscopic examinations, we were able to demonstrate a 29% increase in area/height ratio (p = .01; Fig 6). We used the comparative ratio, as this allowed for direct comparison between the different magnifications seen on stroboscopic examination. These differences are due to the tilt of the 70° scope at the time of stroboscopy.

DISCUSSION

The impetus for this series was the poor voice

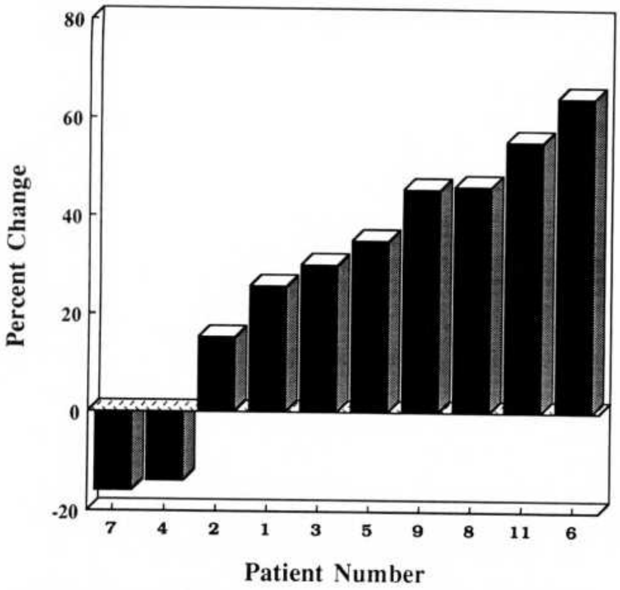

Fig 6. Graph depicting percent change of effective glottic width from preoperative values to postoperative values.

quality results that we achieved endoscopically in most patients undergoing treatment for granuloma. The dysphonic voice of the Teflon patient is created by the mass effect of the granuloma perturbing the relationship of the cover and body and resulting in stiffness and damping.[16] Dysphonic voice is also related to the unequal medialization of the vocal fold. The lateral laryngotomy and reconstruction procedure was designed to account for these pathophysiologic principles.

The initial patients in this series presented with extensive granulomas and firm, fixed, overmedialized vocal folds. Unsure of the long-term voice potential of this procedure, we started with young patients with granulomas so severe that they were facing a lifetime of recurrent treatment for their granuloma. In fact, the entire vocal ligament, as well as the overlying mucosa, was completely resected in 3 of the first 4 patients. Over the first 6 months, all 3 developed significant breathiness with lateralization of the sternohyoid reconstruction. With subsequent fat injection and medialization laryngoplasty, 1 of the 3 has had significant improvement in voice. The other 2 patients have undergone several procedures as outlined in Table 3 for further voice improvement. One continues to have a breathy voice. The other developed significant voice improvement with two different fat injections, but this improvement waned after the fat was resorbed approximately 6 months postinjection.

As confidence developed in the potential improvement with the procedure, the procedure was offered to patients with less extensive granulomas. Of the remaining 5 patients who underwent resection for granuloma, the vocal ligament was spared in 4, and partially resected and reanastomosed in 1. As one can see from the comparison of Table 2 and Fig 5, the voice results were excellent if the vocal ligament and overlying lamina propria were saved (102% increase in the Vanderbilt Voice Score). The excellent return of voice was not hindered in this small series by loss of the ventricular or subglottic mucosa.

This result attested to the central importance of the vocal ligament and the lamina propria in the production of sound. It was possible to acquire good voice results after the body (thyroarytenoid muscle) of the vocal fold was resected and replaced with the nonfunctional sternohyoid muscle. On postoperative stroboscopic examination, we identified an improved mucosal wave on the reconstructed vocal fold in 5 of the 7 cases in which the ligament was preserved. In this limited sample, we were also able to quantitatively demonstrate the importance of the vocal ligament to the reduction of jitter in postoperative voice. The lack of standardization during the voice acquisition process and the transfer of voice signal between the varied formats accounted for the fact that few acoustic analytic conclusions could be drawn. Too often, background noise affected the results of the acoustic analysis. In order to prevent this consequence in future studies, we have standardized our acquisition technique and improved our equipment. We now record patient voice samples directly onto a digital audiotape at the initial contact and all subsequent evaluations.

Arytenoid adduction and revision medialization laryngoplasty were central to acquiring the voice improvement. Two of the 5 patients with intact vocal ligaments developed lateralization of the sternohyoid reconstruction, resulting in mild breathiness. Secondary medialization laryngoplasty improved their results.

Of the final 2 patients who underwent sternohyoid muscle reconstruction after removal of an oversized and ill-designed silicone implant, 1 had no damage to the vocal ligament and developed very good voice with muscle transfer. The vocal ligament of the second was severely damaged by the implant, which protruded 4 mm across the midline. Despite paraglottic reconstruction and secondary medialization, normal voice quality has not been obtained.

Another concept identified in this series related to the pressed voice with which these patients commonly present. This hyperfunctional voice develops secondary to the granuloma extension across the midline with early contact of the membranous fold. An

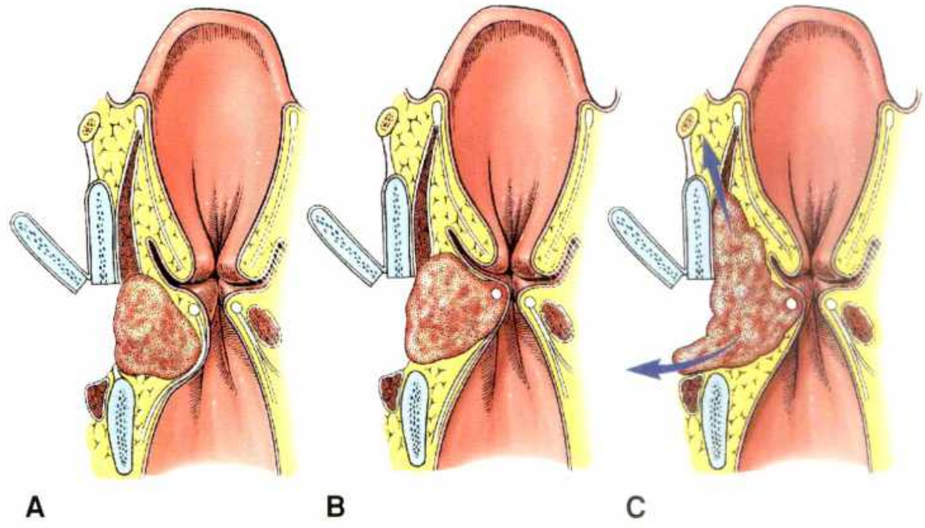

Fig 7. Three possible scenarios for progression of Teflon granuloma. A) Granuloma fills paraglottic space. B) Granuloma involves vocal ligament. C) Granuloma involves vocal ligament and escapes paraglottic space via thyrohyoid or cricothyroid membrane.

oversized silicone implant can result in the same presentation. This extension across the midline also results in slowly increasing airway compromise. By creating a straight vibratory surface on the involved side, we relieved the mechanical obstruction to airflow. At the same time, we were also able to increase the effective glottic width at maximum abduction. Quantitatively, this was demonstrated by the 29% increase in the area/height ratio following the procedure. This procedure and reconstruction then effectively increased the airway size during inspiration and relieved the mechanical obstruction during phonation.

Previously, when significant granuloma formation extended across the midline, endoscopic resection allowed us to improve the airway; however, damage usually occurred to the overlying mucosa and lamina propria. The airway improvement decreased the pressed, strained quality of the voice, but overall, the voice quality was usually very dysphonic and breathy. The exception to this result was the patient with limited granuloma extension into the lamina propria in an isolated location along the membranous vocal fold. This was seen on the endoscopic examination as an isolated focus of stiffness, with mucosal wave formation on either side of the mass effect. A microflap approach with removal of this limited extension often returned the voice to a near-normal quality. Unfortunately, over time, the granuloma was likely to expand again, with the resulting return of dysphonia. Even more potentially damaging was the further growth of the granuloma into the vocal ligament and its lamina propria (Fig 7). Such growth would prevent optimum results of a lateral laryngotomy resection. Further, microflap dissection created scarring, making it more difficult to spare the ligament during an attempt at total resection.

Our experience with the removal of Teflon granuloma pointed to the extensive infiltration of the granuloma into the thyroarytenoid musculature and the paraglottic space. It was difficult to remove the entire granuloma endoscopically. We now feel that two indications exist for endoscopic approaches: 1) microflap exposure with meticulous removal of the medial edge of a limited granuloma extension and 2) gross excision of an extensive granuloma with amputation of the portion of the granuloma crossing the midline and no concern for postoperative voice quality. Moreover, on the basis of the results of this study, we believe that all healthy patients under the age of 50 should be considered for lateral laryngotomy and complete resection of the Teflon granuloma.

The extensive infiltration also contributes to the another common myth related to poor voice created by the granuloma — that is, that the voice can be improved by further medialization of the unaffected segments of the vocal fold. Laryngeal examination in these cases demonstrates that the vocal cords contact early at the location of the granuloma, leaving a gap between the membranous folds posterior to the area of contact. Further medialization of the firm, stiff fold to close the posterior gap results in an increase in the pressed quality of the voice and further dysphonia. One cannot selectively medialize one segment of the fold. Thus, attempts at medialization result in a mass medial displacement of the entire granuloma with disappointing results. Superior results are obtained by complete resection of the stiff granulomatous mass and reconstruction with a supple muscle flap.

CONCLUSION

Teflon granuloma, although an unusual complication, creates a difficult challenge for the reconstructive surgeon. Its infiltration into surrounding tissue makes the preservation of normal structure difficult. Using the lateral laryngotomy technique, one can remove the entire granuloma and create a smooth, straight vibratory surface for phonation. Our results indicate that this technique improves voice, creates a more normal glottic configuration, and decreases airway resistance. Our experience points to the importance of the vocal ligament and the mucosal cover in obtaining good phonosurgical results. Thus, the best candidates for this procedure are patients with granuloma formation with limited involvement of the vocal ligament and sparing of the lamina propria.

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TRAUMATOLOGY OF THE HEAD AND NECK

The Berlin Course on Traumatology of the Head and Neck (with osteosynthesis exercises and live operation) will be held November 5-7, 1998, in Berlin, Germany. The special topic will be acceleration-deceleration trauma (whiplash). For information, contact Prof Arne Ernst, Dept of Otolaryngology at UKB, Rapsweg 55, D-12683 Berlin, Germany; telephone 030 5681 2801; fax 030 5681 2903; e-mail ernst.orl.ukberlin@t-online.de.

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MEYER PROCEDURE FOR SEVERE LARYNGOTRACHEAL STENOSIS

PAUL J. DONALD, MD, FRCS(C)
SACRAMENTO, CALIFORNIA

Many surgical procedures have been devised to manage laryngotracheal stenosis secondary to trauma. Laryngotracheal atresia is the most severe form and the most difficult to repair. The Meyer procedure is a three-stage operation that provides structural support that is covered with mucosa. A laryngotracheal trough is created and a carved trough-shaped cartilage graft is placed above and lateral to it in the first stage. The skin over the graft is replaced by buccal mucosa in the second stage. In the last stage, the cartilage graft with overlying mucosa is swung onto the trough as a composite flap replacing the anterior and lateral laryngeal and tracheal walls. Attempt at reconstruction was made in 8 patients. All but one lesion was secondary to endotracheal intubation. Two patients were unable to be taken to completion of the third stage. Of the remaining 6 patients, all have a functional voice and only 1 remains cannulated at night.

KEY WORDS — cartilage graft, endotracheal tube damage, laryngotracheal atresia, mucosal graft.

Acquired laryngotracheal stenosis and atresia are most often the secondary effect of trauma, and that trauma is most often due to prolonged endotracheal intubation. The resultant lesion produces a severe narrowing and even complete obstruction of the airway, beginning just under the vocal cords and extending through the upper tracheal rings. Of all the reconstructive problems in the head and neck, the most extreme form of such injuries — laryngotracheal atresia — is probably the most difficult to remedy. The existence of multiple techniques to provide a solution for a difficult problem often belies the complete efficacy of any single procedure. This could not be more germane than in the case of laryngotracheal stenosis. In such cases, there are two basic requirements: a rigid skeleton to maintain a lumen and a mucosal lining to provide air humidification. These must then be maintained in a tubelike configuration of adequate diameter to produce a clear airway.

In 1972, Dr Rodolphe Meyer[1] of Lausanne, Switzerland, described a multistage technique for the treatment of this devastating problem. He used a three-stage procedure that supplied a costochondral graft for rigid support and buccal mucosa for lining. A composite flap was created containing these elements and transposed into the defect. He claimed a high degree of success, but this technique has received little attention in North America.

Preoperative evaluation includes direct laryngoscopy and tracheoscopy coupled with computed tomography or, preferably, magnetic resonance imaging of the larynx and trachea to delineate the site and extent of narrowing. Further, an appraisal of the extent of cartilaginous structural loss can be made to anticipate how much support will need to be added.

TECHNIQUE

All patients have a tracheostomy in situ, and the stenosis or atresia begins often just below the level of the vocal cords and extends to just above the tracheostomy site. Surgery is done under general anesthesia by means of a flexible tube through the existing tracheostoma.

Stage 1: Laryngotracheal Trough. A vertical midline skin incision is made from below the hyoid bone to the tracheostomy site (Fig 1[2]). A dissection of the strap muscles is carried out to expose the entire area of stenotic trachea and the skeleton of the larynx above. The stenotic segment is exposed circumferentially to the tracheal-esophageal groove, and the recurrent laryngeal nerves are carefully dissected or are avoided by limiting the lateroposterior dissection. A vertical incision is then made through the cricoid ring and through the involved trachea to the level of the first ring that is not involved in the stenosis (Fig 2[2]). A midline incision through the thyroid cartilage is often necessary as well, with care taken to cut through the anterior commissure precisely. The cricoid cartilage is split posteriorly — if necessary, all the way to the posterior perichondrium, in order to adequately open up the ring. A buccal mucosal graft is inserted into the raw surface produced by the posterior cricoidotomy. Fibrous tissue in the area of the stenosis is dissected completely throughout the involved thyroid, cricoid, and tracheal lumina, with preservation of as much mucosa and cartilaginous structure as possible. There is very little mu-

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From the Department of Otolaryngology–Head and Neck Surgery and the Center for Skull Base Surgery, University of California–Davis, Sacramento, California.

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CORRESPONDENCE — Paul J. Donald, MD, FRCS(C), 2521 Stockton Blvd, #7200, Sacramento, CA 95817.

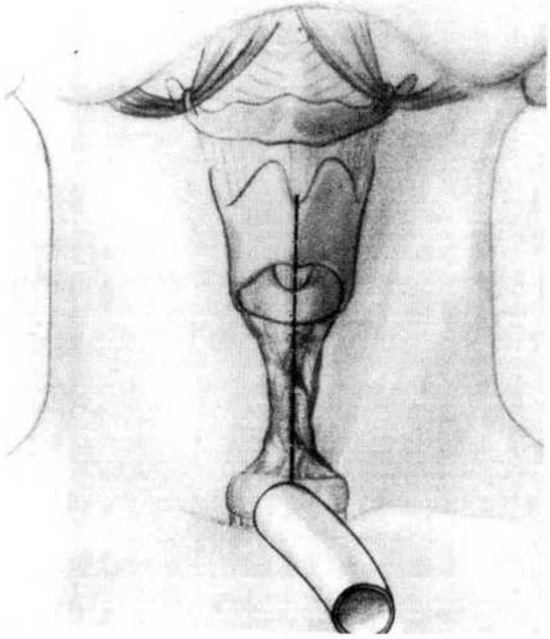

Fig 1. Vertical midline incision. (Reprinted with permission.[2])

cosa in many cases of stenosis, and none in atresia cases. The residual cartilaginous tracheal rings are weakened with numerous cross-hatchings (Fig 3[2]).

Absorbable sutures are now placed to anchor the weakened rings out laterally and are fixed to the deep cervical soft tissue as far laterally as possible. These lateral sutures markedly displace the stenotic walls of the trachea, producing a trough. The residual mucosa of the trachea is sutured to a buccal mucosal graft that is placed against all the raw crosshatched surfaces in the stenotic area. Retention sutures that further assist in holding the weakened rings out laterally are now placed by taking a substantial bite of soft tissue of the weakened tracheal wall and bringing through buttons placed on the external skin surface of the neck (Fig 4[2]). The edges of the skin inci-

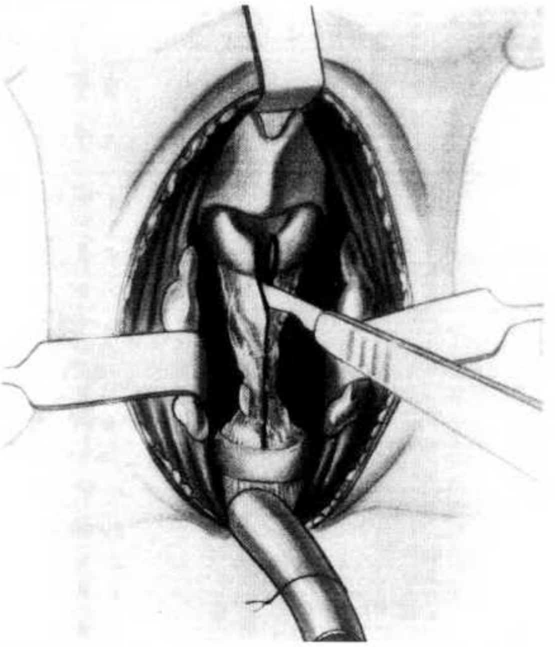

Fig 2. Vertical midline incision made through cricoid cartilage and tracheal rings through length of stenosis. (Reprinted with permission.[2])

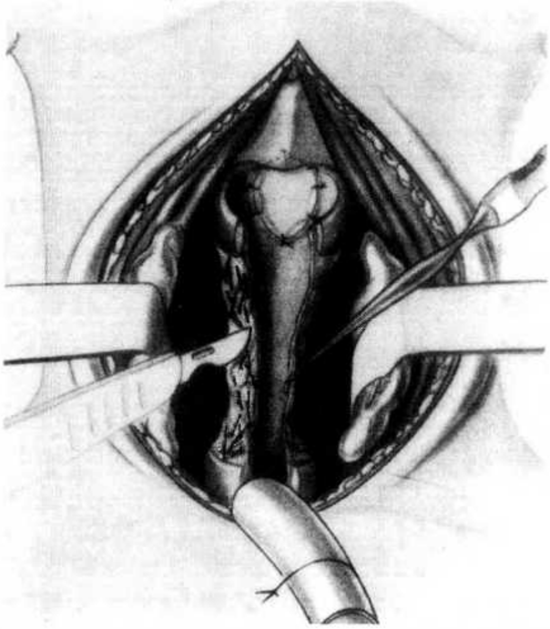

Fig 3. Collapsed, deformed tracheal rings are crosshatched and morselized. Note that posterior cricoid split has been done and buccal mucosal graft placed in defect. (Reprinted with permission.[2])

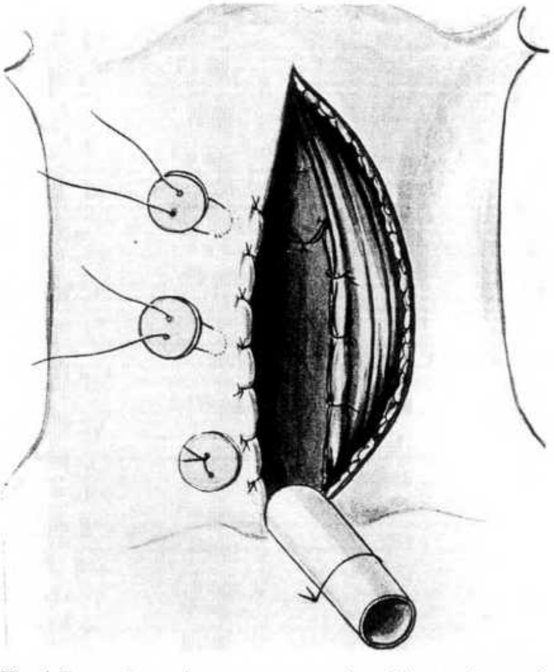

Fig 4. Lateral traction sutures are placed in trachea and cricoid, passed transcutaneously, and anchored to external buttons, thus maintaining trough configuration to larynx and trachea. (Reprinted with permission.[2])

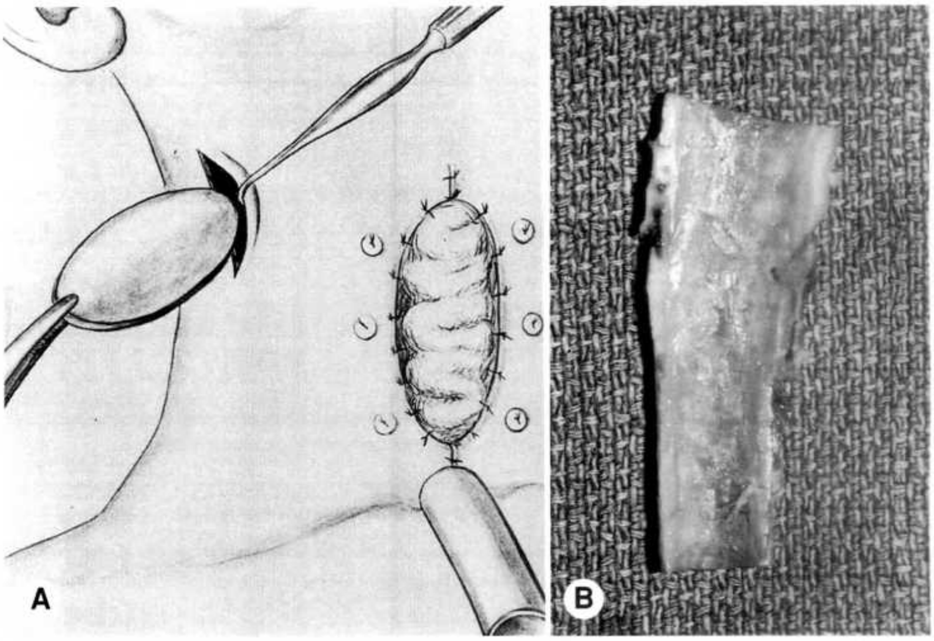

Fig 5. (Reprinted with permission.[2]) A) Costochondral graft carved in trough configuration is placed in subcutaneous pocket. Laryngotracheal trough is packed with medicated gauze. B) Irradiated cartilage graft carved in trough configuration.

sion in the neck are sutured to the mucosa on each side of the laryngotracheal trough, and gauze packing is placed within the trough to hold the mucosal graft against the raw surface and to help further preserve the configuration of the trough by maintaining the lateral position of the walls.

A costochondral irradiated cartilage graft that has been carved in a trough shape is placed in a subcutaneous pocket superiorly and laterally to the trough (Fig 5[2]). The concavity of the trough faces the skin. The skin overlying the cartilage graft is packed into place and a pressure dressing is applied. The tracheostomy is placed below the trough through a separate, horizontally placed skin incision when possible.

Stage 2: Mucosal Graft. A buccal mucosal graft is placed over the cartilage graft 3 weeks following stage 1. The skin over the previously placed cartilage graft is excised just down to the subcutaneous tissue. It is essential to avoid any exposure of the cartilage graft. A full-thickness buccal mucosal graft that will replace the excised skin over the irradiated cartilage graft is harvested. The graft is placed over the cartilage and sutured to the surrounding skin. A medicated gauze bolster is applied to the mucosa to imbricate it into the trough previously carved in the cartilage graft. The previously placed retention buttons holding the morselized cartilage rings out laterally are removed if inflammation is present, or if loosening has occurred.

The packing of the laryngotracheal trough two to three times per day is continued. It is absolutely essential to keep the mucosal graft wet during the interval between this and the final operation. If the buccal mucosal graft sloughs or contracts and becomes too small, then it is excised and replaced with a second graft.

Stage 3: Trough Closure. The closure of the laryngotracheal trough and the reconstruction of the trachea is done 3 to 8 weeks following stage 2. The buccal graft is kept healthy and moist by the frequent application of medicated gauze to the graft surface. The healing of the mucosa to the surrounding skin must be complete and mature at the time of the reconstruction. The chondral mucosal flap is now incised, based upon a random-pattern, horizontally placed soft tissue pedicle that is just above and lateral to the laryngotracheal trough (Fig 6A[2]). Care is taken to keep the pedicle at least as wide as the cartilage graft. The mucocutaneous junction is incised, with great care taken to avoid exposure of the cartilage itself. A generous margin of soft tissue is maintained around the cartilage graft, pedicled on the soft tissue carrier. The chondromucosal flap is transposed and the pedicle is turned 180° such that the mucosal surface faces the lumen of the laryngotracheal trough, and it is sutured into position with absorbable sutures (Fig 6B[2]). A local rotation flap of cervical skin is advanced into the defect for cutaneous closure. An antibiotic dressing is placed on the wound and a pressure dressing is applied for 72 hours.

Stage 4: Inspection and Decannulation. A tracheoscopy is done at 4 to 5 weeks following stage 3 to ensure an adequate tracheal lumen. All granulation tissue is removed with the carbon dioxide laser. Decannulation is done once the tracheostomy has been successfully corked for 2 weeks.

METHODS AND MATERIALS

The records of all patients with severe laryngotra-

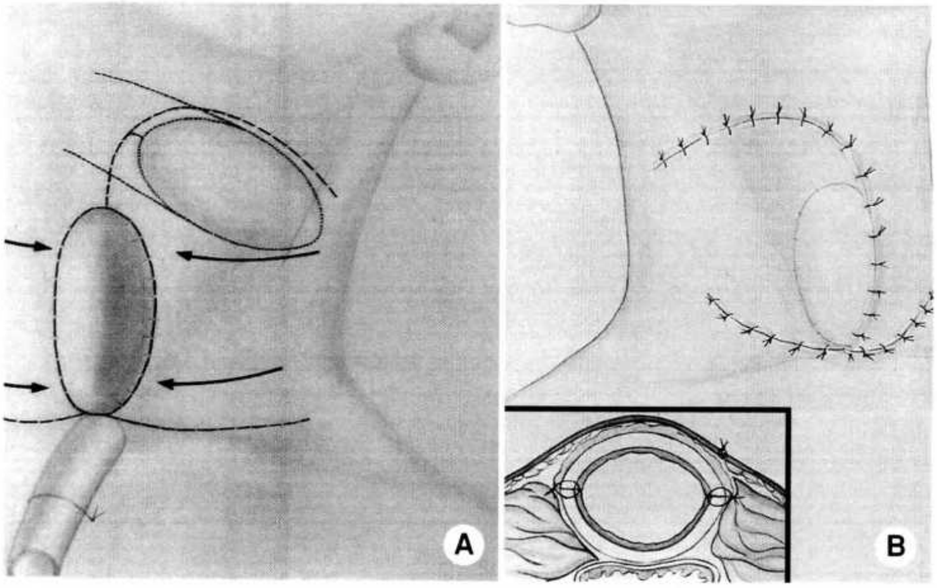

Fig 6. (Reprinted with permission.[2]) A) Medially pedicled composite flap of cartilage trough and mucosal lining is swung 180° into laryngotracheal trough. B) Skin defect closed with local rotation flap. Inset) Cross section showing composite flap in situ with local cervical skin flap rotated into position to cover cutaneous defect.

cheal stenosis or atresia seen at University of California–Davis Medical Center from 1974 to 1996 and treated with the Meyer procedure were reviewed and their cases were analyzed. Data related to the cause, past therapy, and the preoperative degree of stenosis were determined. The operative findings and eventual outcome were tabulated.

RESULTS

There were 8 patients who fell into the category of severe obstruction. All had atresia or tight stenosis from at least the level of the cricoid, extending distally for a minimum of one ring. Those with thyroid stenosis had mainly heavy, thick scar formation that occluded the lumen. All of the patients were hypertrophic scar formers. Seven patients were aphonic prior to surgery, and the eighth had a very poor voice. All but 2 were victims of prolonged endotracheal intubation (Table 1). The duration of intubation varied from 7 to 60 days and averaged 23.5 days. One patient was the victim of a laryngotracheal burn acquired in an automobile racing accident in which he sustained severe flame damage to the larynx and the upper trachea. He was intubated for 2 days and then had a tracheostomy. The ignition of a silicone laryngotracheal stent by subsequent laser treatment resulted in further damage, with burning of the trachea to the carina. On referral to the author, the patient had developed atresia of the larynx from his poorly mobile vocal cords through the subglottis to the carina. Because of subsequent tracheomalacia, he requires a cane tube extending to just above the carina in order to prevent tracheal collapse, and thus, he cannot be decannulated. He has a small residual area of stenosis just below his cartilage graft site that requires laser treatment once a year in order to maintain a satisfactory lumen for speech. Until a few weeks prior to each laser treatment, he maintains a good voice but a marginal airway. Because of his hypertrophic scar–forming tendency, a stage 3 reconstruction has not been possible. He has had two trough procedures, and each time, the trough has filled in with scar before the last stage could be completed.

Prior surgery in attempts to open the airway was performed on 6 patients, with an average number of procedures of three per patient. Prior to the Meyer procedure, 1 patient (patient 6) had seven laser treat-

TABLE 1. PREOPERATIVE DATA

Patient	*Sex*	*Age (y)*	*Cause of Stenosis*	*Duration of Intubation (d)*	*Duration of Stenosis (mo)*	*No. of Prior Operations*
1	M	21	Intubation	30	12	2
2	M	32	Intubation	14	24	3
3	F	55	Intubation	60	14	0
4	F	67	Intubation	30	48	0
5	M	26	Intubation	35	8	1
6	M	15	Intubation	10	24	7 Laser, 1 reconstructive
7	M	38	Fire	2	8	2
8	F	28	Fire	7	0	3

TABLE 2. OPERATIVE FINDINGS AND FOLLOW-UP DATA

Patient	*Level of Stenosis*	*Time From Stage 1 to Stage 3*	*Time From Stage 3 to Decannulation*	*Subsequent Surgery*	*Follow-up*	*Postoperative Voice**	*Remarks*
1	Thyroid, cricoid, 2 tracheal rings	8 wk	12 wk	None	18 y	Moderately hoarse	
2	Cricoid, 2 tracheal rings	6 wk	6 wk	None	8 y	Normal	
3	4 Tracheal rings	24 wk	Decannulated at stage 3	None	1 mo	Slightly hoarse	Short neck, no tracheostomy
4	Thyroid, cricoid, 1 tracheal ring	0†	Failure	None	6 y	None	
5	Thyroid, cricoid, 1 tracheal ring	12 wk	Tracheostomy tube at night	Multiple laser procedures above stoma	5 y	Moderately hoarse	1 Paralyzed vocal cord, 1 paretic vocal cord
6	Cricoid, 2 tracheal rings	16 wk	15 wk	None	6 y	Normal	
7	Thyroid to carina	0†	Never decannulated	Yearly laser procedures	10 y	Moderately hoarse	
8	Cricoid, 2 tracheal rings	12 wk	16 wk	4 Laser procedures	5 y	Mildly hoarse	Postoperatively, 1 laser procedure for web, 3 laser procedures for squamous epithelium

*Only patient 2 had voice preoperatively, with severe hoarseness.

†Stage 3 not completed.

ments and one reconstructive procedure, and another (patient 8) had three major reconstructive procedures. Decannulation after the Meyer procedure was successful in 5 of the 8 patients (62.5%). A successful voice was achieved in 7 of the 8 (87.5%). One patient (patient 3) had such a short neck that no tracheostomy was done at stage 3. Although she had an excellent voice and a good airway, she died of a stroke 1 month after surgery. She had two strokes some years prior, with good recovery from both. In the 4 other patients who were decannulated, the average time to decannulation was 12 weeks. One patient (patient 5) in the unsuccessful decannulation group goes without a tracheostomy tube during the day, but wears the tube at night. He has a paralyzed vocal cord on one side and a weak cord on the opposing side. Following arytenoidectomy, he has a good voice but a marginal airway. Another patient who was a failure of decannulation is the only one who is aphonic. She acquired laryngotracheal atresia following prolonged intubation after open heart surgery that was preceded in turn by a number of cardiac arrests. She was also a hypertrophic scar former. Because of her prosthetic cardiac valve, she was on anticoagulation therapy. Her postoperative course was plagued with recurring hematomata at the reconstruction site. This resulted in subsequent fibrosis compromising her repair, and her tracheal atresia has recurred.

DISCUSSION

The literature is replete with reconstructive techniques designed to remedy laryngotracheal stenosis. The use of these procedures in minor degrees of stenosis has a high incidence of success, but in major ones it has a higher failure rate. A common thread is obvious in those patients who have severe stenosis or atresia, namely, collapse or loss of tracheal rings and cricoid cartilage and minimal or absent mucosa. Their airways require a rigid support that will maintain a tubular airway of adequate dimension and an epithelial lining that will humidify the air and impede subsequent obstruction by fibrosis.

Numerous materials to provide the rigid support necessary for the larynx and trachea have been suggested, such as bone, cartilage, and a host of alloplastic materials including a number of plastic and metallic meshes. With the emergence of metallic plate splints, the mesh grafts appear to have been abandoned. In the recent literature, techniques appear to be divided into the following categories: 1) resection of the cricoid arch and trachea with thyrotracheal anastomosis, 2) cartilaginous graft interposition, 3) some form of pedicled muscular flap, 4) vascularized free flaps, 5) muscle and titanium plates or some form of ceramic, and 6) periosteal or perichondrial flaps.

The largest series of patients was described by

Grillo et al,[3] and included 503 patients who had 521 tracheal resections with reconstruction. This included 62 patients who had sustained subglottic injury as well. In the laryngotracheal cases, the anterior arch of the cricoid was resected and a tracheal thyroid anastomosis was completed as primary treatment. In the group of tracheal and combined laryngotracheal injuries that could be followed up (471), there was an 89.5% success rate. In those that had subglottic and tracheal resection repaired with a cricotracheal anastomosis, a "good" or "satisfactory" result occurred in 90.3% of cases. The number of cases with atresia was not mentioned. Maddaus et al[4] reported 53 cases of tracheal stenosis that included 15 cases of severe circumferential laryngotracheal stenosis. These patients were treated in a fashion similar to that in Grillo's series, with circumferential tracheal resection, partial cricoidectomy, and a tracheothyroid anastomosis. Extubation was successful in 51 of 53 patients. However, in the group of 15 with severe laryngotracheal stenosis, laryngofissure was also performed. Thirteen of the 15 have been decannulated. The voice was described as satisfactory to good. Macchiarini et al[5] stated that the above-described procedure should be done for tracheal and subglottic stenosis extending to only within 1 cm of the vocal cords. Their series of 26 patients were seen over a 10-year period. The stenoses were all the result of intubation. They had a 96% success rate, with only 6 patients receiving a tracheostomy in the postoperative period, and those all being extubated within 24 hours of the tracheostomy. Since tracheostomy was necessary in nearly 25% of their patients, prophylactic tracheostomy would seem to have been a wise option in all of them.

In another series by Grillo et al,[6] 80 cases of upper tracheal and subglottic stenosis were repaired by anterior cricoid arch resection, removal of the subglottic scar, preservation of the posterior cricoid plate, and then coverage of the residual cricoid with a membranous posterior tracheal wall flap. A cricotracheal and thyrotracheal anastomosis completed the repair. Results were reported as excellent in 18, good in 48, satisfactory in 8, and failure in 2. Only the failures required persistent intubation. The satisfactory patients had hoarse voices and some stridor.

Chen[7] did an experiment on six dogs with a "rotary door" sternohyoid myocutaneous flap that was stiffened by a costal cartilage graft. At sacrifice, the five surviving animals had an excellent tracheal lumen without stricture, the cartilage was incorporated into the framework, and the tracheal lumen was covered by normal respiratory mucosa. Sixteen patients with laryngotracheal stenosis were operated upon with this technique, and all were successfully decannulated with "normal" exercise tolerance. Friedman and Mayer[8] reported on an 8-year series of tracheal and severe laryngotracheal stenoses repaired with a sternocleidomastoid flap and attached clavicular periosteum. The periosteum provided a source for potential bone formation. There were 26 patients in the series, and 25 have been decannulated. One patient had sternal osteomyelitis and mediastinitis, and another required revision.

In 1995 and 1996, Esclamado and colleagues[9,10] used a pedicled perichondrial flap in rabbits for laryngotracheal reconstruction. The vascularized perichondrium underwent chondrogenesis and retained a semblance of tracheal configuration and was not influenced favorably or adversely by the placement of a buccal mucosal graft or stent.

Weisberger and Nguyen[11] placed either a fascia graft or auricular cartilage in 13 patients with laryngeal and tracheal stenosis. The cartilage was supported by a Vitallium miniplate. The plate provided an external brace while respiratory mucosa migrated over the graft. They had a 77% success rate with an airway sufficient to permit decannulation. Follow-up averaged 32 months. Time from surgery to decannulation was short, and there were no instances of infection or plate extrusion. However, in an experimental series reported by Mitskavich et al,[12] in which a cricoid and tracheal split was produced in a growing larynx in piglets, half of the microplates placed migrated into the laryngeal lumen.

Casiano et al[13] reported a 78% (7/9) success rate in using a neurovascularized strap muscle flap to fill the laryngotracheal defect with the cartilage remnants splinted apart by a titanium miniplate. Their patients had 60% to 100% stenosis preoperatively, with 3 having 100% stenosis. The muscle was reepithelialized in 3 weeks. Two plates underwent extrusion. Decannulation was successful in 2 of the 3 patients who had 100% stenosis.

An experimental study by Triglia et al[14] compared use of a ceramic hydroxyapatite splint to the standard method of laryngotracheoplasty with costal cartilage. A high degree of absorption of both types of grafts was encountered — worse in the hydroxyapatite graft group than in the cartilage group.

The most popular interposition graft used to provide tracheal cartilaginous support is autogenous cartilage. The problems with autogenous cartilage grafts center around their unpredictable propensity for warping and absorption.[15] Irradiated cartilage, when placed in sites that are non–stress-bearing or against which no pressure is exerted, survives well in humans, despite poor success in animals.[16] Cartilage has carvability, which enables the surgeon to fashion a graft with a troughlike configuration. Rib, iliac crest, and calvarial bone, although more rigid, lack this property. Alloplastic materials have the inherent problem of being unable to sustain a mucosal graft. Meshed alloplastic materials implanted in the neck will allow nutrition to the mucosal graft to pass through their interstices. However, the mesh is very close to the surface, and the potential for exposure and subsequent extrusion is great.

Ideally, the lining of the subglottic larynx and the trachea would be of respiratory epithelium. Unfortunately, there are few opportunities for graft sites that include ciliated epithelium. Skin grafts have been used in the past, but are dry and desquamate; thus, patients are plagued by a chronic cough productive of secondarily infected squamous debris. This was a particular problem in my patient 8, who had only small strips of skin that were included on the edges of the mucosal graft. One solution to the problem of supplying a rigid support with an attached, wettable lining was provided by Watson et al,[17] who reconstructed the trachea with a vascularized free flap of rib and attached pleura. Curiously, the pleural epithelial lining underwent a metaplasia from a serosa to a respiratory epithelium. Although successful in dogs, this technique has not been attempted in humans.

Unfortunately, in virtually all experimental animal studies the greatest flaw in design is the artificial creation of a laryngotracheal defect that the test procedure is used to reconstruct. There has not been a study in which a real stenosis has been created and then subsequently reconstructed. The presence of deformed, contracted, fragmented tracheal rings and cricoid cartilage; fibrosis; and scarred, depleted mucosa in the experimental animals is necessary to adequately reproduce the problem encountered in humans. The problem of comparison of the efficacy of the various procedures described in the literature with the Meyer procedure, as described here, is that all of my patients but one had total atresia of the subglottic and tracheal lumen and were hypertrophic scar formers. The atresia in my cases involved the cricoid and sometimes the thyroid cartilage, and usually at least the upper two tracheal rings. It is difficult to glean from the literature[3-14] how many of the described patients had atresia or stenosis of this severity. I reserved the Meyer procedure for cases in which single-stage procedures had either failed or did not appear to be feasible. Regarding the 2 patients who were not given an initial single-stage procedure, patient 3 had an extremely short neck, and primary anastomosis would not have been feasible, and patient 4 had the trachea, cricoid, and thyroid cartilage involved.

Currently, single-stage reconstruction with a thyrotracheal anastomosis is the procedure of choice. In hypertrophic scar formers, the circular closure that is inherent in this procedure presents an opportunity for cicatrix. In instances in which the thyroid cartilage is scarred inward or prior reconstructive procedures have failed, the Meyer procedure, although staged and prolonged, provides a good option. The Meyer technique provides an excellent solution to the severely stenotic and atretic upper airway. A rigid trough of cartilage is lined with a mucus-secreting epithelium. The drawbacks to the procedure are the necessity of multiple stages and the scarring of the neck. Decannulation was successful in 5 of the 8 patients here described. On the other hand, the restoration of voice has been successful in all but 1 patient. The remaining 2 patients that have not been decannulated have other significant problems with the airway: in one, a tracheomalacia from the stoma to the carina, and in the other, a paralyzed cord on one side and a paretic cord on the other side. The Meyer procedure appears to be an effective method of reconstruction of the most difficult cases of upper airway atresia and stenosis.

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RECORDING FROM AFFERENTS IN THE INTACT RECURRENT LARYNGEAL NERVE DURING RESPIRATION AND VOCALIZATION

KEITH F. CLARK, MS, MD JAY P. FARBER, PHD

OKLAHOMA CITY, OKLAHOMA

The goal of this study was to determine whether sensory fibers in an intact recurrent laryngeal nerve (RLN) are influenced by respiration or vocalization. Patterns of RLN afferent activity were examined during respiration and evoked vocalization by means of midbrain electrical stimulation in cats anesthetized with α-chloralose. Nerve bundles were dissected from an intact RLN, with motor function preserved. The bundles were cut and the laryngeal end was placed on floating bipolar electrodes. Fifteen right RLNs were examined. A total of 9 single and multiunit afferent fibers from 4 cats were isolated and examined during respiration. Four units, analyzed from 3 fibers, showed respiratory phase modulation. Eight units, analyzed from 4 fibers in 1 cat, were observed during vocalization and showed no vocalization phase modulation. The RLN afferents could contribute to reflex modulation of the respiratory cycle, but more extensive sampling would be necessary to preclude effects from vocalization.

KEY WORDS — cat, proprioception, recurrent laryngeal nerve, reflexes, respiration, superior laryngeal nerve, vocalization.

INTRODUCTION

Indirect anatomic and electrophysiologic evidence suggests that the few sensory fibers present in the laryngeal motor nerves — the recurrent laryngeal nerve (RLN) and the external branch of the superior laryngeal nerve (eSLN) — might contribute to a reflex system controlling intrinsic laryngeal muscles during respiration and vocalization.[1-8] Sensory information from laryngeal mucosa, joints, and other deep structures is carried by these motor nerves. The eSLN provides innervation to the mucosa of the anterior subglottis and cricothyroid membrane,[8] as well as the cricoarytenoid joints.[9] Recurrent laryngeal nerve afferent fibers have been traced histologically to the cricothyroid joint,[4] the lateral subglottic mucosa,[8] and the undersurface of the vocal folds.[3] Mechanical stimulation of mechanoreceptors in the laryngeal joint capsules elicits reflexes in the intrinsic laryngeal muscles; this finding suggests that joint receptor output contributes to normal laryngeal coordination.[4,9] Subglottic nerve endings responded in physiologic tests to the types of stimuli that occur during respiration and phonation: touch, puffs of air, passive movements of the larynx,[1,6] and vibratory stimuli.[6] Slowly adapting responses similar to those due to muscle spindles have also been recorded from afferent fibers in the RLN of cats; this finding suggests that the larynx is provided with a stretch-receptor mechanism.[2] Although muscle spindles have been identified in all of the intrinsic muscles of human larynges,[10] the larynges of cats do not have muscle spindle organs.[11] There are other specialized nerve endings found in intrinsic laryngeal muscles that *may* assume the proprioceptor's role. The highest density of muscle spindles in human thyroarytenoid muscle is located near the free margin,[10] and this superficial vocalis portion has the most sensory innervation.[12] "Spiral nerve endings" have been described coiled around cat[13] and human[11] muscle fibers concentrated in this superficial area at the medial edge of the vocalis muscle in the zone of motor innervation. These were thought to represent a primitive form of muscle spindle organ.

It is possible that the sensory information carried by the RLN and eSLN may influence respiration and vocalization. However, the basic question of how mechanoreceptors, served by sensory fibers in the laryngeal motor nerves, respond during respiration and vocalization remains unanswered. The purpose of this project was to identify and categorize patterns of mechanosensitive responses in sensory fibers within the two motor nerves, the RLN and the eSLN, during evoked vocalization by using midbrain electrical stimulation in cats anesthetized with α-chloralose. These patterns of activity could then be contrasted to those found by this laboratory in the major sensory nerve, the internal branch of the superior laryngeal nerve (iSLN). Because we wanted

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From the Departments of Otorhinolaryngology (Clark) and Physiology (Farber), University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma. Supported by the American Laryngological Association, the Presbyterian Health Foundation (PHF #771), and the National Institutes of Health/National Institute on Deafness and Other Communication Disorders (R03-DC-01201-01A1). This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Oklahoma Health Sciences Center.

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CORRESPONDENCE — Keith F. Clark, MS, MD, Dept of Otorhinolaryngology, PO Box 26901, Oklahoma City, OK 73190.

these afferents to be exposed to normal stimuli produced by laryngeal muscle contractions, a technique was developed whereby small bundles of the RLN and eSLN were microdissected, cut, and placed on electrodes while leaving the main trunk, and vocal fold mobility, intact. During whole nerve transection, used by other investigators, mechanoreceptors might be silenced or activity otherwise eliminated by vocal fold paralysis. Afferent unit and multiunit activity was isolated from the cut laryngeal end of these small bundles by using floating bipolar electrodes that retained their contact with nerve fibers despite movement due to breathing and phonation.

METHODS

Anesthesia. Cats were first tranquilized with intramuscular ketamine hydrochloride (10 mg/kg). Then 1% Xylocaine with 1:100,000 epinephrine was injected into the groin, followed by placement of femoral vein and artery catheters for drug administration and blood pressure monitoring, respectively. An initial loading dose of α-chloralose was given intravenously that was typically 75% of the final dose. Additional boluses were given over a 1.5- to 3-hour time period to a dose of 40 to 60 mg/kg. This protocol was used to maximize the effectiveness of chloralose while allowing the ketamine effect to dissipate. When the limb withdrawal reflex and pupillary reaction to paw pinch became consistently absent and blood pressure stability occurred, then anesthesia was considered to be complete. Care was taken to provide complete anesthesia prior to surgical manipulation. Additional anesthetic was given during the experiment as necessary to maintain anesthesia. Blood pressure was recorded with a pressure transducer connected to the femoral artery catheter. Rectal temperature was controlled (37°C ± 1°C) by an automatic heating blanket. At the end of the experiment, animals were euthanized by pentobarbital sodium overdose (>200 mg/kg).

Vocalization. Anesthetized cats were placed in a stereotaxic device. A craniotomy was prepared to position a stimulating electrode at stereotaxic coordinates of zero in the anterior-posterior plane and 1.5 mm lateral in the medial-lateral plane, and then the electrode was positioned in the dorsal-ventral plane at 0.5-mm increments to the range of 1.5 cm above the interaural plane until the strongest phonation was produced with the least stimulating current. These coordinates would be expected to place the electrode tip in the region of the caudal midbrain periaqueductal gray,[14] but the position of the electrode tip was not confirmed histologically. Stimulating parameters of 1 to 3 mA, 200 microsecond pulse duration, at 100 Hz were used to produce vocalizations.

Nerve Dissection. A neck incision was made and the skin was used to contain a pool of warm mineral oil (39°C). The RLN was exposed in the right tracheoesophageal groove inferior to the larynx. The eSLN was exposed just proximal to the point at which it penetrated the cricothyroid muscle after removing the overlying strap muscles and separating it from the iSLN. A technique was developed whereby small bundles of the RLN and eSLN were microdissected and cut while leaving the main trunk and vocal fold mobility intact. The laryngeal end of the cut bundle was then teased apart and small slips of nerve (fibers) were placed over the electrodes until action potentials (spikes) from a single unit or several units (multiunit) were present. Additional attempts were made at isolation of viable eSLN nerve fibers by using the whole nerve transection technique, which involved teasing apart fibers from the transected nerve. Both microdissection and whole nerve transection were also applied to the iSLN to compare the effectiveness of the techniques in isolating viable single fibers.

A 1-cm-square steel plate was used to mount bipolar recording electrodes; it was cemented to the tracheal wall or laryngeal skeleton with cyano-acrylate cement. The electrode pair, which had been attached with epoxy to a small beryllium magnet, was held tightly to the steel plate, but allowed electrode position adjustments to be made. The electrode configuration allowed the electrodes to move with the nerve and helped prevent the nerve from being pulled off the electrode complex when the animal swallowed, coughed, or vocalized. The tip of a flexible fiberoptic endoscope (Olympus EnFP-2, Hitschfel Instruments Inc, St Louis, Mo) with an attached video camera was passed transorally and suspended inside the hypopharynx above the laryngeal inlet to allow monitoring of vocal fold mobility by direct observation.

Peripheral Nerve Recording. Action potentials were high-pass fourth-order filtered at 20 Hz, amplified with a preamplifier (World Precision Instruments, model DAM-5A, Sarasota, Fla), displayed on an oscilloscope and audio monitor, and then digitally recorded at 22 kHz.

Recording Phonation. Acoustic data were acquired via a custom-built amplifier and microphone (Bruel and Kjaer, model UA0196, Copenhagen, Denmark) placed 15 cm from the mouth. Output from the microphone was passed to the oscilloscope and digitally recorded at 22 kHz. Identification of onset and offset of phonation was determined from the acoustic (voice) channel.

Electromyography. Electromyography (EMG) ac-

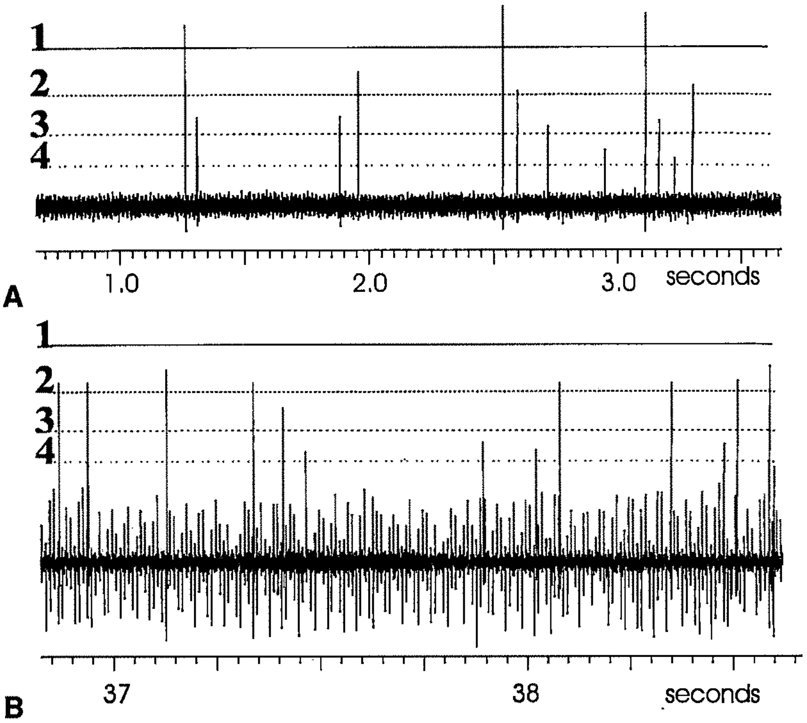

Fig 1. Short segment of raw record from multiunit fiber is presented here to demonstrate how amplitude is used to differentiate units. Additional details of this fiber's response to respiration and vocalization are depicted in Fig 6. A) Control respiration. B) Vocalization. A shows 4 single units active in this fiber. Four voltage amplitude zones demarcated by 4 horizontal broken lines consistently identify each unit over time. During vocalization sequence, contact of electrodes with fiber changed due to movements of electrode. Amplitude of all spikes increased, but relative amplitude differences were apparently maintained. Either highest-amplitude spike in A is missing in B, or lowest-amplitude unit has become obscured by stimulus artifact. Low-amplitude activity in B near baseline is due to electrical stimulus used to evoke vocalization.

tivity of the diaphragm and cricothyroid muscles was monitored by bipolar electrodes placed into the respective muscles. The costal diaphragm muscle was exposed through a midline skin incision over the xiphoid process. The two hook-wire needle electrodes were passed just behind the xiphoid process, parallel to the plane of the diaphragm muscles, under direct vision. This placement minimized contamination of the EMG by intercostal muscle activity and was confirmed by timing of diaphragm EMG. The electrodes were placed into the cricothyroid muscle under direct vision. Each EMG was amplified (Grass model 7DAG, West Warwick, RI), viewed on the oscilloscope, and digitally recorded at 10 kHz. The onset of diaphragm EMG defined the onset of inspiration. Declining EMG defined the onset of expiration. Cricothyroid EMG was monitored during dissection of the eSLN and iSLN to ensure the proper branches were selected and to determine that dissection of the SLN did not produce cricothyroid paralysis. We did not record from the thyroarytenoid muscle, to avoid activation of RLN afferents by the mechanical effects of the recording electrode itself.

DATA ANALYSIS

Individual fiber action potentials (spikes) were identified by visual inspection of the printed record. In each case, the spike activity of an isolated nerve fiber was recorded during both respiration and vocalization. Voltage amplitude, spike shape, and regularity of firing rate were used to distinguish spikes from the electrical stimulus used to evoke vocalization and other artifacts (Fig 1). Changes in spike rate across phases were compiled manually. Phases included 1) inspiration and expiration during spontaneous respiration and 2) inspiration, expiration, and phonation during periaqueductal gray stimulation (vocalization). In most cases, the discharge pattern and rate were variable over the course of recording. This either was due to the response of the nerve or was caused by technical aspects of the microdissection technique. We selected areas of the recording that appeared to have activity that could be analyzed. Computer-assisted automatic selection using amplitude was done for 2 fibers that did have constant activity throughout the recording. In these cases it was possible to determine instantaneous mean frequency (ImeanFr) to graphically indicate the fiber's spike rate across phases. The ImeanFr is the reciprocal of the time between two spikes. The ImeanFr tracing displayed spike-to-spike changes in firing rate. The majority of fibers had very low spike rates, and ImeanFr tracing was not used in these cases.

RESULTS

The whole nerve transection technique was technically easier to perform on all nerves studied. The iSLN served as a control to determine how dissection technique might influence yield. The iSLN was easier to dissect and tease apart than either motor nerve. The yield of viable fibers from the iSLN with the whole nerve transection technique was 4 times that obtained when the microdissection technique was used. No afferent activity during respiration or

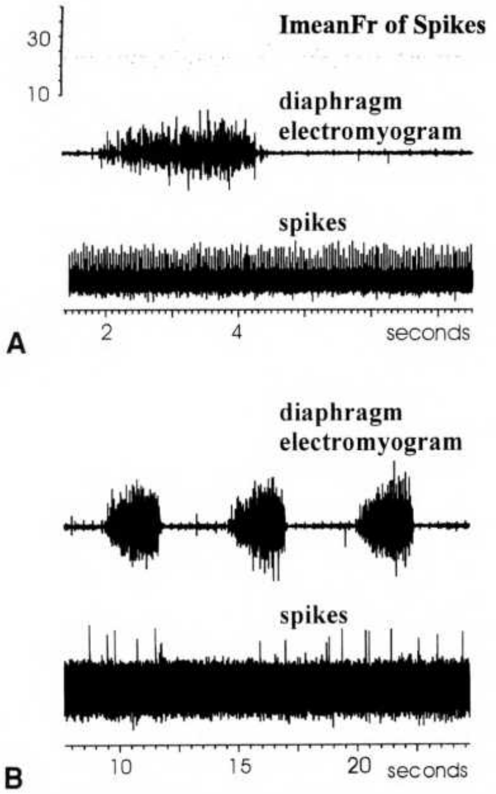

Fig 2. A) Tonic, single unit (rate 23 Hz) with no change during control respiratory phases. ImeanFr of spikes — instantaneous mean frequency of spike activity depicted on bottom channel. B) Example of slow, variable-rate, multiunit fiber with no respiratory modulation.

vocalization was identified in this study from the eSLN by using either the microdissection or whole nerve transection technique on 8 nerves. A total of 9 RLN fibers containing single and multiunit activity were found in 4 cats with the microdissection technique. The firing rate of 2 tonic RLN fibers identified was 20 to 30 Hz. The majority of the remaining fibers discharged less than 5 times over an interval of 1 second.

The behavior of 5 fibers could only be characterized during respiration and not during vocalization, since the fibers were pulled from the recording electrode at the initiation of periaqueductal gray stimulation. Two of these displayed activity that was not influenced by respiratory phase; 1 single-unit fiber, depicted in Fig 2A, had a tonic spontaneous rate of 23 Hz, and 1 multiunit fiber had infrequent spike activity, as seen in Fig 2B. Two fibers evidenced inspiratory modulation; 1 single-unit fiber paused at the onset of inspiration and then resumed firing until a tonic rate of 20 Hz was reached by the end of inspiration, which was maintained across expiration (Fig 3). A second, multiunit fiber contained 2 units that exhibited respiratory modulation (Fig 4).

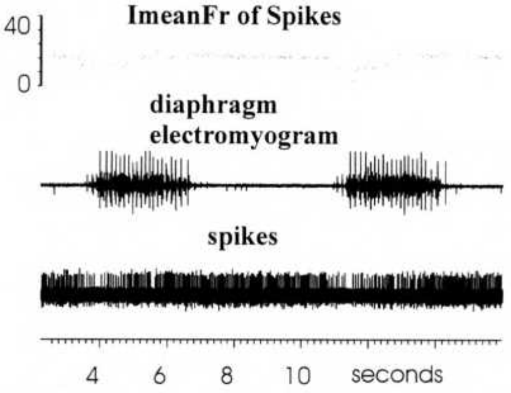

Fig 3. Example of single unit showing decrease in activity at beginning of inspiration. ImeanFr of spikes — instantaneous mean frequency of spike activity shown on bottom channel.

The activity of 4 fibers from 1 cat was examined during both respiration and evoked vocalization sequences. Figure 5 displays a rarely active single-unit fiber with 4 of 5 spikes occurring during early expiration when diaphragm muscle activity was declining. Although this fiber shows expiration modulation, it remained silent during many inspiratory and expiratory phases during both control respiration and vocalization sequences. Two units were found in another fiber that remained quiet except for a pair of spikes during the final phonation of the vocalization sequence. The third fiber was quiet during spontaneous respiration but had an inconsistent, brief, low-rate bursting pattern during 3 of 4 inspirations during vocalization. It also responded after the midbrain stimulation was initiated during 2 expirations before the cat started to phonate and did respond during 1 phonation, but was silent during most of the recording. The activity of the fourth, multiunit fiber, containing 4 units (Figs 1 and 6), likewise did not show consistent modulation by phases of respiration or vocalization, although there was some increase in spike rate during the vocalization sequence (Fig 6).

DISCUSSION

The goal of this project was to characterize the activity of sensory fibers carried in the laryngeal motor nerves during respiration and vocalization. Suzuki and Kirchner[6] suggested a role for RLN afferents in a reflex system of control during vocalization based on the sensitivity of subglottic receptors to touch, airflow, vibration, and the observed reflex effects on laryngeal muscles in response to subglottic touch and electrical stimulation of the RLN. We sought to test this hypothesis. Unfortunately, we were only able to successfully collect data from a limited number of RLN afferents. Our data could not confirm a potential role for RLN afferent information in the control of vocalization, since very little activation of RLN afferents was seen during phonation. Our ability to

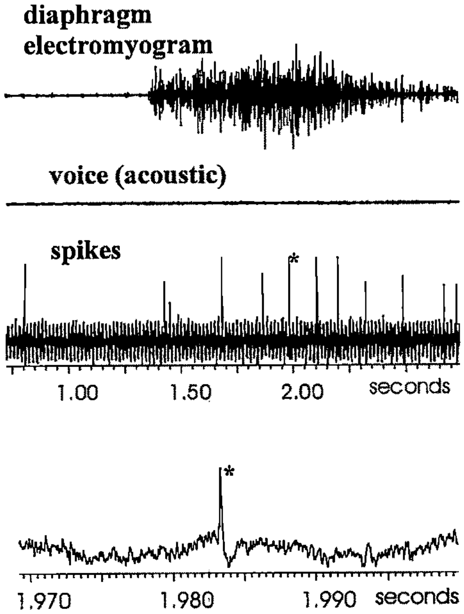

Fig 4. One respiratory cycle is depicted during which multiunit activity is shown with at least 2 units activated during inspiration. Channel labeled "spikes" is expanded in lower panel to allow visualization of shape of action potential marked by asterisk.

successfully collect data from a limited number of RLN afferents may explain our failure to identify a potential role for RLN afferent information during vocalization. In addition, it was only possible to dissect from the lateral aspect of the RLN, with the microdissection technique; this may also have decreased the yield of afferent fibers. These results are in contrast to the activity seen in this laboratory among fibers in the iSLN during electrically evoked vocalization, where 85% were expiratory-modulated and 37% of these followed the vibratory pattern of the vocal folds.[15] We are unable to explain the difference between our degree of success with recording afferents in the eSLN and that reported by Suzuki and Kirchner,[5] who found "a large proportion" of afferent fibers in the cut eSLN using electrophysiological recording techniques. This may be due to differences in which branches of the nerve were considered to constitute the eSLN. Since the eSLN joins the iSLN, one could include or exclude fibers depending on where the two were separated. In a histologic study using wheat germ agglutinin–horseradish peroxidase injections into the nodose ganglion of cats, no sensory fibers were labeled in the eSLN.[16] These authors were also unable to resolve this difference.

The present study represents the first report of activity of RLN afferents recorded from an intact nerve with vocal fold movement intact. With this model we were able to identify both inspiratory and expiratory modulation of some RLN fibers during respiration. Others have identified the presence of sensory fiber activity but have not appreciated respiratory phase modulation, presumably due to vocal fold paralysis.[1,3,6] In a similar manner, afferents in the iSLN show a high degree of respiratory modulation that is apparently related to vocal fold movements, since many lose this characteristic after ipsilateral RLN section.[3,17,18] This concept is further supported by the finding that many iSLN fibers retain respiratory modulation, when vocal fold movements are intact, even when airflow is diverted away from the larynx via tracheotomy with balloon or catheter

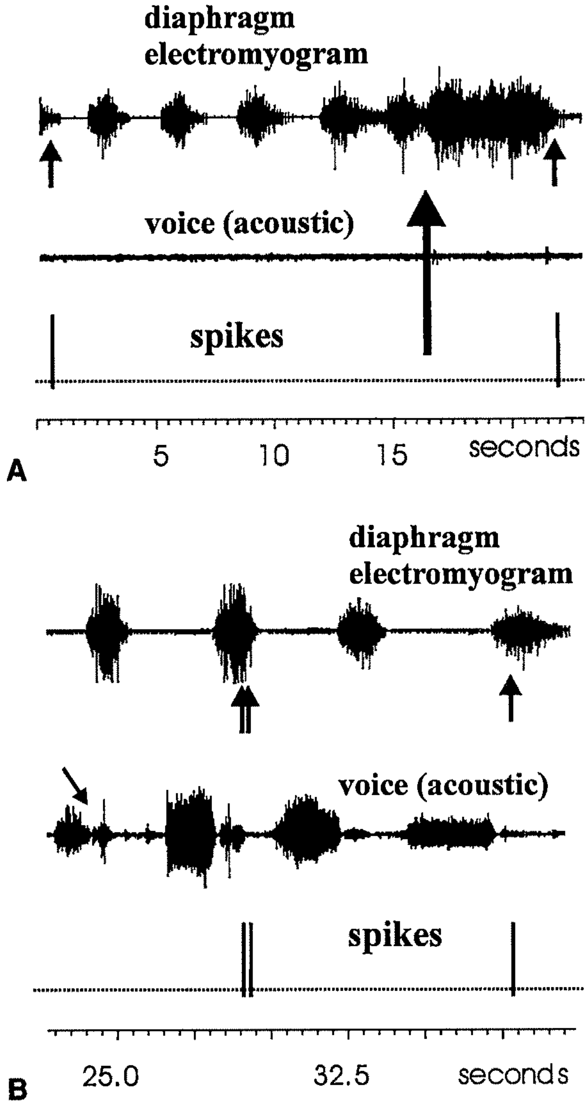

Fig 5. Spikes from this example were identified by inspection and depicted by vertical bar. A) Control respiration. B) Vocalization. Although this single-unit fiber only responded with 5 spikes during this 36-second recording, 4 of 5 spikes seen on this single-unit record occurred during early expiration while diaphragm activity declined. Alignment of spike to diaphragm tracing is accentuated by vertical arrows. Large vertical arrow near 16 seconds in A indicates onset of periaqueductal gray stimulus. Angled arrow on voice channel in B shows recording of audible sound during inspiration.

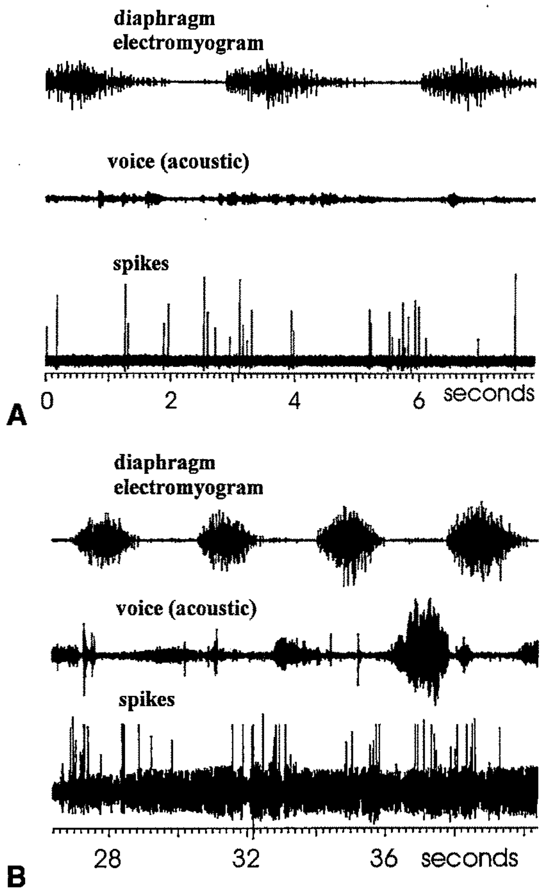

Fig 6. Response of this multiunit fiber is shown during portion of A) control respiration and B) vocalization. Four single units can be distinguished during control respirations (also see Fig 1). Multiunit activity during vocalization appears to increase overall, but there is no consistent relationship to vocalization.

occlusion of the upper trachea.[18,19] These and other findings have led investigators to suggest a role for iSLN afferents in reflex control of breathing and airway patency.[18,20] Although we did not test responses to positive or negative pressure as described by Mathew et al[21-23] (due to the difficulty of sealing the mouth and nose with the stereotaxic device and fiberoptic scope in place), the finding of modulation by respiratory phase supports a similar role for RLN afferents.

Results of our study are apparently consistent with the rarity of afferent fibers in the laryngeal motor nerves. The RLN has been described as a "unimodal" nerve with little histologic evidence for sensory fibers on the basis of axon diameter.[24,25] Later studies contradict this concept by describing a small proportion of RLN fibers of appropriate diameter for proprioception in humans and cats,[7] in spite of the absence of muscle spindles in cat laryngeal muscles. These large fibers account for approximately 2% of nerve fibers in cat RLNs.[7] Large (10 to 15 μm) myelinated fibers are known to innervate proprioceptors (muscle spindle organs) in other muscles of the body.[26,27] The low proportion of afferents in the RLN likely contributed to the low numbers of successfully isolated fibers in the present study. Since human larynges are provided with muscle spindles, human RLN afferents may play a more important role.

The RLN and eSLN were more difficult to dissect than the iSLN, presumably due to connective tissue content differences. The microdissection technique decreased the yield, since only fibers on the lateral aspect of the RLN were dissected. From the work of Gacek and Lyon,[7] the afferents appear to be randomly distributed across the nerve at the level of the upper trachea. Thus, one would have to cut across the entire nerve in order to have a chance of sampling all afferents. In addition, it is difficult to detach a bundle of fibers from the intact nerve without killing the included fibers. This was illustrated by significantly lower yields when the microdissection technique was applied to the iSLN as compared to the whole nerve transection technique. Videoendoscopy was used to determine if there was a gross paralysis of the vocal fold. It is likely that we could not visualize the subtle motor deficit that occurred as a result of the microdissection technique used to isolate fibers. This might be an additional factor limiting the yield of fibers activated during phonation, but would not eliminate the response of mucosal or joint receptors.

Hirose[1] was able to successfully record from only one type of afferent in the RLN after RLN transection. These receptors were thought to reside in the fascia or connective tissue and were not muscle spindles. They only responded to separation or compression of the laryngotracheal junction and showed no change in activity after section of the opposite RLN and both SLNs. The fiber depicted in Fig 2A shares the characteristics of discharge rate and lack of respiratory modulation with the type of afferent reported by Hirose. It is possible that Hirose did not see respiratory phase modulation because he transected the RLN. The response of afferents to respiratory phase may be caused by vocal fold movement.[3,17,18] The fiber shown in Fig 3 has a similar tonic rate and could, on this basis, be a similar fiber showing inspiratory modulation. In our protocol, inspiratory modulation was preserved, since the vocal fold was not paralyzed. We were unable to manipulate the laryngotracheal junction in this case.

Kirchner and Wyke[4] described RLN responses to cricothyroid joint capsule stretch. Rapidly adapting fiber activity from receptors has been found in all the laryngeal joints of cats.[4,12] Brief bursts of activity occurred at the onset of movements. Release of a displaced joint caused a second brief burst. The fiber shown in Fig 5 shows a rapidly adapting response pattern during some early expiratory phases. This might represent a cricothyroid joint receptor as described by Kirchner and Wyke.[4]

Sampson and Eyzaguirre[3] found fibers with slowly adapting responses in the RLN on tapping the undersurface of the vocal folds. These receptors were described as being below the base of the vocal folds. Suzuki and Kirchner[6] noted subglottic receptors that were slowly adapting and that responded to light touch and a small jet of air. We were unable to probe subglottic mucosa, since this requires laryngofissure, but expected these receptors to respond during expiration or phonation, yet we could see no evidence for activation of this type of receptor. Other receptors found by Suzuki and Kirchner[6] that responded to heavier probing also followed a vibrating stimulus. Several investigators have noted the ability of iSLN laryngeal touch receptors to follow vibratory stimuli.[3,28] We could not find evidence of RLN receptors that followed the vibratory pattern of the vocal folds during phonation. Such vibration-following receptors are common in the iSLN.[15] Again, the lack of fibers responding to pressure, flow, or vibration could be explained by our inability to identify enough afferents using the microdissection technique.

The goal of this study was to determine whether sensory fibers in the RLN are influenced by respiration or vocalization. The number of afferents examined during respiration and vocalization was admittedly very small, due to the difficulty of keeping the nerve fibers in contact with the recording electrode, and due to the problems discussed related to dissecting a slip of nerve from the lateral surface of the intact RLN. Other limitations may have been imposed by electrically evoked phonation and by the lack of a method to characterize fibers based on response to mechanical stimulation or pressure change before vocalization. From this small sample, we were not able to support the hypothesis that RLN afferents are influenced by vocalization; however, RLN afferents could contribute to reflex modulation of the respiratory cycle. Future improvements in our ability to record from afferent fibers in laryngeal motor nerves without disturbing motor function are needed in order to make conclusions about the relevance of afferent information carried by them.

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MENIERE'S DISEASE

The Fourth International Symposium on Meniere's Disease will be held in Paris, France, April 11-14, 1999. For information, contact Administrative and Scientific Secretariat, SOCFI/MENIERE '99, 14 rue Mandar, 75002 Paris, France; telephone 33 (0) 1 44 88 25 25; fax 33 (0) 1 40 26 04 44; e-mail fournier@socfi.fr.

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Ann Otol Rhinol Laryngol 107:1998

EFFECT OF PENICILLIN ON FORMATION OF FIBROUS ADHESIONS IN ACUTE OTITIS MEDIA

PER CAYÉ-THOMASEN, MD MIRKO TOS, PHD

COPENHAGEN, DENMARK

Fibrous middle ear adhesions are occasionally encountered in middle ear surgery and may cause a hearing impairment. Although usually associated with chronic otitis media, adhesions are also found following a single episode of experimental acute suppurative otitis media, suggesting a pathogenesis based on the inflammatory process engaging acute infection. In a well-established rat model of pneumococcal acute otitis media, we report on the effect of penicillin V on formation of fibrous middle ear adhesions. Previous studies have shown marked impact of penicillin on mucosal goblet cell density and other histopathologic features. Number, anatomic localization, and histopathologic morphology of adhesions were assessed in a longitudinal study of 25 normal, 25 untreated, and 25 treated rats. Although penicillin administration induced a slight tendency toward fewer ears with adhesions and fewer adhesions per ear, these changes were nonsignificant. Histomorphology and the general pattern of anatomic localization of adhesions were unaffected by penicillin administration. We conclude that administration of penicillin has an inconspicuous effect on the formation of fibrous adhesions in experimental acute otitis media caused by *Streptococcus pneumoniae*.

KEY WORDS — histopathology, infection, middle ear mucosa, rat, *Streptococcus pneumoniae*.

INTRODUCTION

Fibrous mucosal adhesions are occasionally found during surgical exploration of the middle ear cavity, usually in chronic otitis media. The adhesions may cause a conductive hearing loss by a fibrous fixation of the tympanic membrane or the ossicular chain,[1,2] and are likely to obstruct mucociliary clearance and hamper optimal ventilation of the middle ear cleft. Further, a successful outcome of surgery to improve hearing may be compromised by adhesions.[3]

Although fibrous adhesions usually are associated with chronic middle ear disease,[1-3] they are also found following a single episode of experimental acute suppurative otitis media (AOM).[4] Accordingly, dynamic mucosal changes induced by the inflammatory reaction accompanying acute infection have been proposed as the pathogenetic basis of adhesion formation in the middle ear.[4]

The mucoperiosteal lining of the middle ear is subject to quite extensive changes during and following experimental AOM.[4-7] The secretory capacity of the mucosa increases, suggesting a correlation to secretory otitis media[5]; polyps and adhesions are formed[4,6]; and the epithelial lining undergoes additional transient and long-standing morphologic alterations.[5,7] Administration of penicillin during experimental AOM decreases mucosal secretory capacity, conceivably preventing a subsequent development of secretory otitis media.[8] A number of other mucosal changes are prevented by penicillin,[9] whereas the effect on polyp formation is inconspicuous.[10]

The effect of penicillin on adhesion formation was investigated in this study with our well-established rat model for AOM, which in its clinical aspects is comparable with AOM in the child.[11] The self-limited infection is induced by inoculation of *Streptococcus pneumoniae*, which causes 30% to 50% of cases of AOM in humans,[12] and lasts approximately 10 days. In contrast to other animals previously used in experimental AOM, rats do develop spontaneous AOM[13] and are not prone to the development of a possibly fatal sepsis during pneumococcal infection.[14] Further, among rodents, the anatomy and drainage conditions of the rat middle ear have the highest comparability with those of the human infant and child.[15-17]

MATERIALS AND METHODS

Twenty-five healthy male Sprague-Dawley rats weighing 200 to 250 g were used. They were kept under standard laboratory conditions and given food (pellets) and water ad libitum. During anesthesia, induced by a short-acting barbiturate (methohexital; Brietal, Lilly, Sweden) administered through a tail vein, a ventral midline incision was made in the neck. With an operating microscope (Olympus, LRI Instruments, Sweden), the right tympanic bulla was ex-

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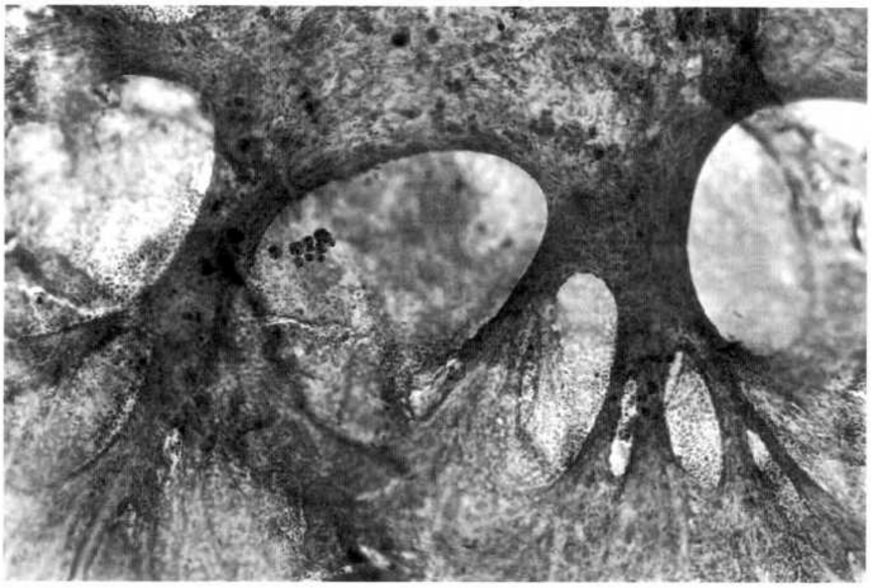
From the Department of Otorhinolaryngology, Gentofte University Hospital of Copenhagen, Hellerup, Denmark. Supported by The Rosalie Petersen Foundation.

CORRESPONDENCE — Per Cayé-Thomasen, MD, Dept of Otorhinolaryngology, Gentofte University Hospital of Copenhagen, DK-2900 Hellerup, Denmark.



Surface view of postinfection rat middle ear mucosa with branched fibrous adhesions (periodic acid–Schiff–Alcian blue, original ×200).

posed and inoculated through the bone with 0.05 mL of a suspension of log-phase type 3 *S pneumoniae* prepared as previously described.[18] The bacteria had been proven sensitive to penicillin in earlier studies.[8,9,19] The core of the pneumococcal suspension was determined as 5×10^6 colony forming units per milliliter. Two days after inoculation, all rats were given penicillin V (Guttae Calciopen 250 mg/mL) 100 mg/kg body weight orally, as 1 daily dose for 5 days. On days 4, 8, and 16 and 3 and 6 months after inoculation, 5 rats were randomly selected and painlessly sacrificed, on each occasion by intracardiac perfusion with formol-alcohol (1 part 40% formaldehyde, 2 parts 96% alcohol). The perfusion lasted 10 minutes, whereafter the rats were decapitated and the heads transferred into vials for post-fixation by immersion in the same fixative for at least 1 week. Rats sacrificed at 3 and 6 months were examined otomicroscopically every 14 days to rule out spontaneous infection, and all rats were examined immediately before sacrifice. The inoculated middle ear bullae were removed, opened, and divided into 2 halves, followed by periodic acid–Schiff (PAS)–Alcian blue staining. The mucosa was carefully dissected from the bone by means of a stereomicroscope (model 570, American Optical Corporation, Buffalo, NY), and the stained mucosa was placed wholemount in a chamber filled with anise oil–colophonium (a mixture of anise oil 19 g, colophonium 20 g, and kinin-chloride 5 g), sealed with paraffin (Tissue-wax, Pro-Hosp, Vaerløse, Denmark). The mucosa was carefully placed in the chamber according to its anatomic location in the middle ear bulla. The number and anatomic localization of mucosal adhesions were registered in a light microscope (Olympus BH-2, Biko A/S, Denmark). Mucosal parts containing adhesions were serial-cut and stained with a combination of the PAS–Alcian blue and hematoxylin-eosin methods. The histomorphology of adhesions and the surrounding mucosa was registered. Results were compared with those of 25 normal and 25 untreated, inoculated middle ears, prepared and studied exactly as described above, as previously reported.[4] The Mann-Whitney test was used for statistical analysis, with $p < .05$ as the critical level of significance.

RESULTS

Whole Mounts. The normal ears contained no adhesions. In untreated animals all ears from day 8 and later contained adhesions (see Figure), whereas 2 ears from penicillin-treated animals were without adhesions. The median number of adhesions per ear and the maximum number for the group were nonsignificantly but slightly lower in treated animals (Table 1). Treatment did not affect the anatomic sites of predilection for the development of adhesions, which were the hypotympanum, followed by the anterior epitympanum, the attic, the eardrum, the interossicular spaces, and the tubal orifice. However, adhesions attached to the promontory were found in a treated animal, but not in untreated animals (Table 2).

As did untreated animals, treated animals displayed frequent branching of the middle ear adhesions (see Figure) and increased goblet cell density in the area beneath and around the adhesions, as compared to surrounding parts of the mucosa.

Sections. Serial sectioning of the middle ear mucosa from treated animals showed epithelial hyperplasia in the areas beneath and around the adhesions, which were covered with single-layered, predominantly squamous (flat) — in places, cuboidal — epithelium, with or without goblet cells. Subepithelial

TABLE 1. NUMBER OF FIBROUS ADHESIONS PER MIDDLE EAR FOLLOWING INDUCTION OF ACUTE OTITIS MEDIA IN FIFTY EARS

		Days After Inoculation				
		4	*8*	*16*	*90*	*180*
Untreated ears	Median	0	11	17	14	15
	Range		3-21	5-29	2-29	5-20
Treated ears	Median	0	10	14	14	13
	Range		4-19	4-25	0-30	3-22

glands were seen in the mucosa beneath and around the adhesions. These findings were similar to findings in untreated animals.

DISCUSSION

The formation of fibrous adhesions in AOM may be perceived as a dynamic process of mucosal alterations encompassing 6 pathogenic stages: 1) localized rupture in epithelium and basal membrane, 2) prolapse of lamina propria through rupture, 3) epithelialization of prolapsed lamina propria, 4) growth and elongation of epithelialized prolapse, and 5) fusion of the tip or end of the prolapse with another part of the mucosa, leading to 6) formation of an adhesion.[4]

This study showed that the administration of penicillin during experimental AOM has little or no preventive effect on the formation of fibrous mucosal adhesions, as only a slight tendency toward fewer ears with adhesions and a lower number of adhesions per ear was found. The histopathologic findings revealed no qualitative differences in adhesions or the immediate mucosal surroundings in middle ears of treated versus untreated animals. The general pattern of anatomic localization of the adhesions was unaffected by penicillin administration, albeit a few adhesions attached to the promontory were found in a treated animal.

Considering that previous investigations have shown a marked impact of penicillin on clinical observations and histomorphology of the middle ear mucosa in AOM,[8,9,19] the impact on adhesion formation seems surprisingly modest. However, assuming that local tissue trauma is the initial event in the pathogenesis of both polyps and adhesions,[4,6] the present findings are concordant with the insignificant effect of penicillin on the formation of mucosal polyps.[10] The local tissue trauma inflicted on the middle ear mucosa during the acute phase of infection is seen as localized ruptures of the epithelium and basement membrane,[6] caused by a number of interplaying factors and events of the inflammatory process. Among these are a generalized mucosal edema, liquid exudation (intraepithelial and subepithelial liquid accumulation), passage through the epithelium of luminally migrating granulocytes, and the release of a wide array of cytotoxic agents, eg, granulocyte enzymes and oxygen-derived free radicals.[6,20-23]

As the severity of the above events and the quantity of the inflammatory mediators released during the acute phase of the infection are diminished by administration of the bacteriocidic penicillin, a considerable diminution of local tissue trauma and subsequent adhesion formation would seem plausible. On the other hand, the expression of inflammatory mediators is well under way and early mucosal changes are already present prior to antibiotic intervention on day 2.[19,24] Further, the inflammatory response may persist, due to the presence of bacterial cell wall products, as well as to the continuation of the inflammatory cascade initiated at inoculation.[25,26]

Treatment was initiated on day 2 in order to mimic the clinical situation. On day 2, otomicroscopy revealed dilated blood vessels over the malleus and a beginning accumulation of purulent effusion in the middle ear.[19] Effusion was not found on day 1 and is, in a majority of cases, the sign that initiates antibiotic treatment in Scandinavia. Intervention on day 2 might have been too late to influence the abovementioned events leading to adhesion formation,

TABLE 2. NUMBER OF MIDDLE EARS WITH POSTINFECTION ADHESIONS IN VARIOUS LOCATIONS

	To						
From	*Eardrum*	*Ossicles*	*Promontory*	*Hypotympanum*	*Attic*	*Anterior Epitympanum*	*Tubal Orifice*
Eardrum							
Ossicles	2/1						
Promontory	0/1	0/1					
Hypotympanum	5/3	1/0		18/16			
Attic	3/3	4/5			9/7		
Anterior epitympanum	2/0	1/0			2/0	10/11	
Tubal orifice				2/3		1/0	

Number before slash is number of untreated middle ears; number after slash is that of treated middle ears. "From" and "to" portions together indicate middle ear locations that adhesions ran between.

considering the time lapse from oral administration to the peak mucosal concentration of the penicillin. Further, the number of colony forming units in the bacterial inocula might have been so high that the penicillin only insufficiently reduced the number of bacteria in the acute phase, thus insufficiently suppressing the noxious stimuli leading to the initial epithelial ruptures.

Moreover, although epithelial ruptures are frequent, the development of an adhesion from a rupture is probably not mandatory. Reparative processes of the epithelium probably furnish full mucosal regeneration from the majority of ruptures, leaving the mucosa without adhesive sequelae. Thus, a relatively high number of epithelial ruptures might only yield a limited number of adhesions. Accordingly, penicillin administration may limit the number of ruptures, but only diminish the number of adhesions inconspicuously.

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MUCOCELE OF THE SPHENOID SINUS: A LATE COMPLICATION OF TRANSSPHENOIDAL PITUITARY SURGERY

PHILIPPE HERMAN, MD, PHD
RÉMI MARIANOWSKI, MD
GUILLAUME LOT, MD
MICHEL ASSAYAG, MD
JEAN-PIERRE GUICHARD, MD
PATRICE TRAN BA HUY, MD
PARIS, FRANCE

In an 18-month period, 3 cases of sphenoidal mucocele following pituitary surgery were diagnosed at our institution. Only 1 case of this late-onset disorder has yet been reported as a pitfall of the transsphenoidal route. Symptoms include recurrent headache and visual complications. Diagnosis was delayed because of misinterpretation of the magnetic resonance imaging findings, which actually showed the development of a sphenoid mucocele long before clinical symptoms occurred. These 3 cases suggest that attention should be focused not only on the sella turcica, but also on the sphenoid sinus, in analyzing the magnetic resonance imaging data. The treatment consists of endoscopic transnasal marsupialization, since the mucocele is lined by normal epithelium with an inflammatory reaction that will heal with drainage. At the time of surgery, prevention would require either endoscopic control of the mucosal remnants, in case of sinus exclusion, or leaving the sphenoid sinus air-filled under a sealed sella.

KEY WORDS — headache, hypophysectomy, mucocele, pituitary surgery, sphenoid, transsphenoidal surgery.

INTRODUCTION

Most pituitary tumors are operated on via the transsphenoidal route. The use of the transsphenoidal approach goes back to the inception of modern neurosurgery. Harvey Cushing described in 1909 the transseptal transsphenoidal approach to the sella turcica as a rapid midline route. Since then, the transsphenoidal approaches have become standard practice.[1-3] Postoperative radiologic follow-up is routinely performed after removal of pituitary tumors 1) when residual tumor is suspected at surgery, 2) when persistent or recurrent hypersecretion indicates a residual or recurring hormonally active adenoma, or 3) in the case of nonsecreting pituitary adenomas. However, interpretation of the magnetic resonance imaging (MRI) data may be hampered by misunderstanding of the changes induced by surgery.

To the best of our knowledge, this is the second report of sinus mucoceles following pituitary surgery.[4] Diagnosis was delayed because of misinterpretation concerning the MRI findings, which actually showed the development of a mucocele long before clinical symptoms occurred. These 3 cases suggest that attention should be focused not only on the sella turcica, but also on the operative area, when analyzing the MRI findings.

CASE REPORTS

Case 1. A 30-year-old woman presented 8 years earlier with clinical symptoms of acute pituitary apoplexy. Computed tomography (CT) revealed a large pituitary tumor with suprasellar extension. The tumor was successfully removed by a transseptal transsphenoidal approach, which resulted in permanent panhypopituitarism requiring replacement therapy. Because of extensive necrosis, immunocytochemistry could not be performed, and the final diagnosis was that of a nonsecreting adenoma. The sphenoid sinus was packed with abdominal fat. One year later, the patient complained of recurrent, severe headaches with nausea that lasted 2 to 3 days. The symptoms worsened with time, requiring several hospital admissions. When MRI was performed, the radiologist mentioned postoperative changes of the sphenoid sinus and no recurrence of tumor. Seven years after surgery, the radiologic findings (Fig 1) were reconsidered because of the onset of an incomplete nerve III palsy. The intense hypersignal on T1- and T2-weighted sequences, with an irregular increase in intensity of the sinus lining, suggested the presence of a typical mucocele rather than fat or muscle in the sphenoid. Computed tomography showed a bony bridge between the sphenoid sinus and the nasal cavity. Endoscopic decompression of the mucocele was performed through a transethmoidal approach and provided rapid relief of the symptoms. One year later, the patient remained symptom-free. Endoscopic examination revealed a large and permeable sphenoidotomy.

Case 2. A 64-year-old woman was admitted to the emergency department of our institution with a se-

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From the Departments of Otolaryngology (Herman, Marianowski, Tran Ba Huy), Neurosurgery (Lot), Neuroradiology (Guichard), and Endocrinology (Assayag), Hôpital Lariboisière, Paris, France.

CORRESPONDENCE — Philippe Herman, MD, PhD, Hôpital Lariboisière, Service ORL, 2, rue Ambroise Paré, 75010 Paris, France.

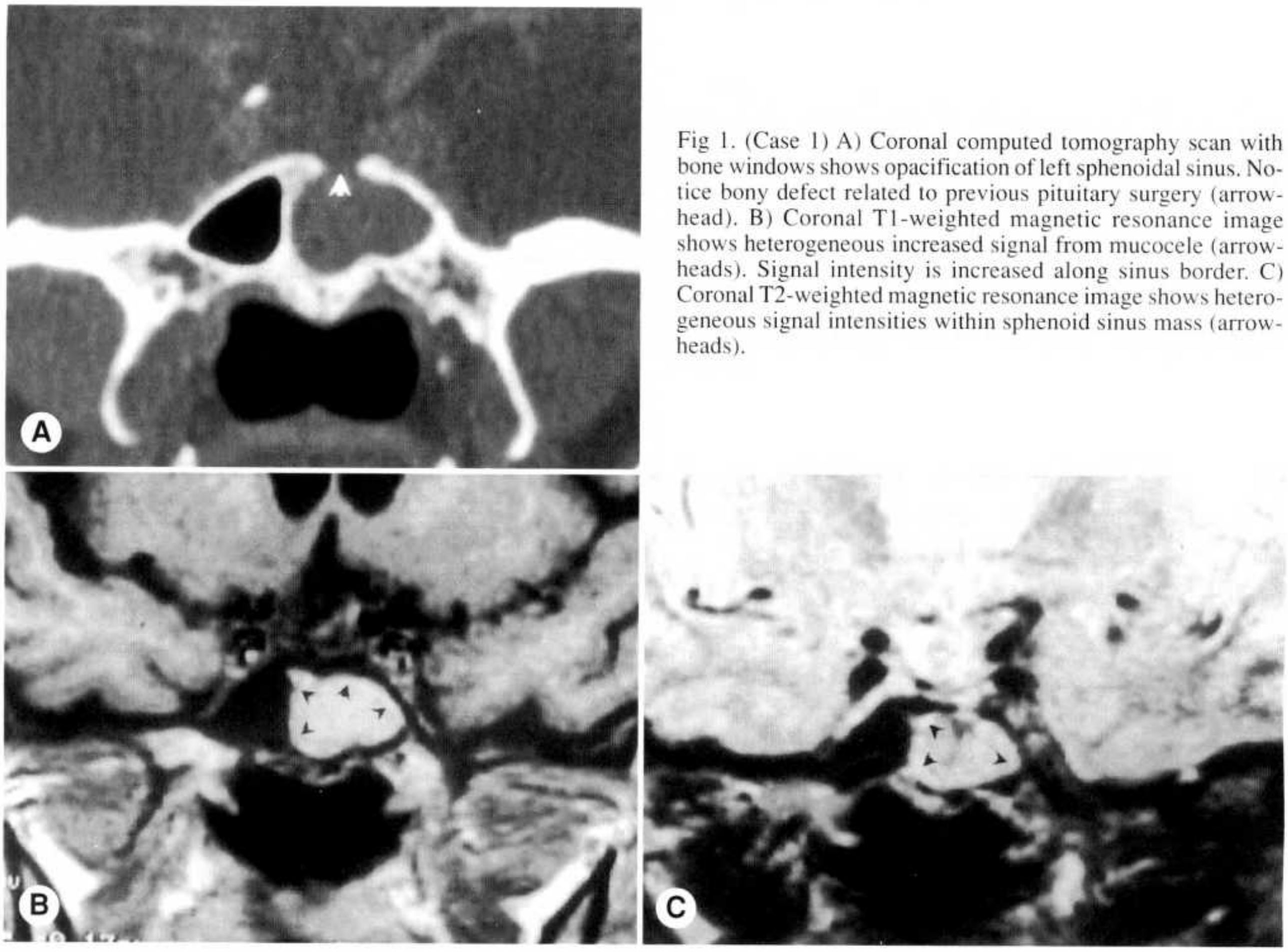

Fig 1. (Case 1) A) Coronal computed tomography scan with bone windows shows opacification of left sphenoidal sinus. Notice bony defect related to previous pituitary surgery (arrowhead). B) Coronal T1-weighted magnetic resonance image shows heterogeneous increased signal from mucocele (arrowheads). Signal intensity is increased along sinus border. C) Coronal T2-weighted magnetic resonance image shows heterogeneous signal intensities within sphenoid sinus mass (arrowheads).

vere headache, followed by nausea, vomiting, and acute loss of visual acuity. A large sellar and suprasellar tumor was diagnosed, and the symptoms were attributed to pituitary apoplexy. Ophthalmologic examination revealed a left lateral hemianopsia and a right visual loss (sense of light). Transsphenoidal surgery was performed, which allowed the complete removal of the tumor, which turned out to be a necrosed nonsecreting adenoma. Hormonal replacement therapy was given and the hemianopsia recovered, while the vision of the right eye remained poor. One year later, she began to suffer twice a year from recurrent attacks of headache with nausea. Each crisis was violent, and lasted 24 hours. Multiple investigations were performed. Magnetic resonance imaging showed a complete obliteration of the sphenoid sinus, with a signal compatible with either a recurrence of the adenoma or a mucocele. The radiologic findings remained stable over time, and reintervention was postponed for 2 years. Finally, increasing headache led to endoscopic surgery. The superior-posterior part of the nasal cavity was found to be obstructed by thick fibrous tissue. Removal of the latter revealed a cystic cavity filled with xanthochromic mucoid material, which was aspirated. A large sphenotomy was performed in the anterior wall of the sphenoid sinus. Immediate relief of headache was observed, and follow-up remained uneventful for 18 months.

Case 3. A 51-year-old diabetic woman was treated 15 years earlier at another institution by pituitary surgery for a nonsecreting adenoma. The follow-up was uneventful and she did not require replacement therapy. The need for increasing doses of insulin and the occurrence of a biologic inflammatory syndrome led to CT of the paranasal sinus, which evinced a complete opacification of the left sphenoid sinus. Endoscopic examination was unable to show the ostium of the sinus. When MRI was performed, there was a hypersignal in the sinus on T1- and T2-weighted images, with a reinforcement of the signal at the periphery of the sinus.

A surgical endoscopic approach was performed through a transnasal route after removal of the middle turbinate (Fig 2A). In the sphenoethmoidal recess, a bulging of the mucosa was seen at the site of the ostium of the sphenoid sinus. After incision of the mucocele and aspiration of a viscous mucus, a large sphenotomy was performed. The diabetes could then

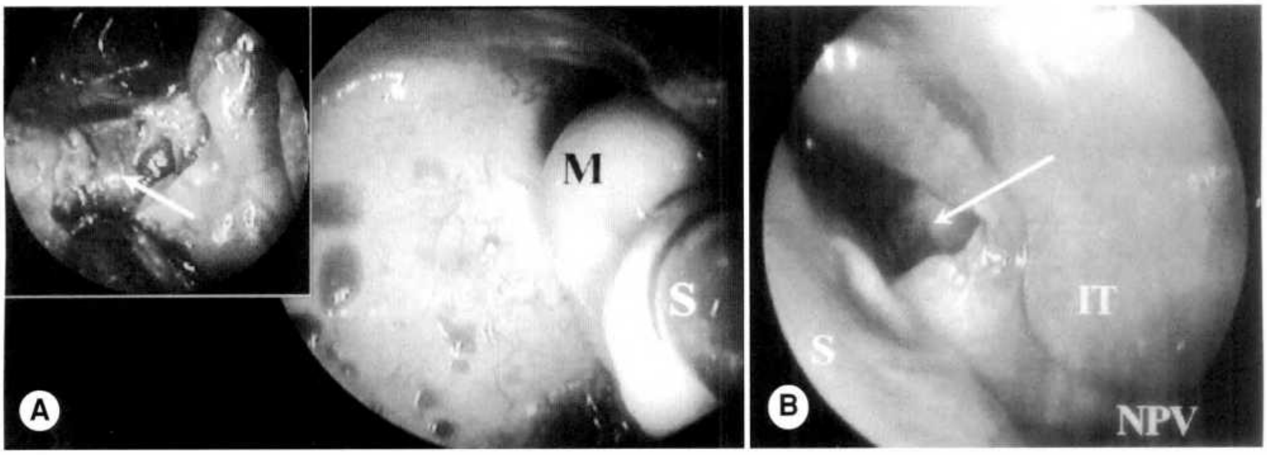

Fig 2. (Case 3) A) Endoscopic transnasal marsupialization of sphenoid mass. Mucocele is lined by thick mucosal wall (arrow on inset). After incision, viscous mucoid material (M) is aspirated (S). Mucosal lining of sinus has normal appearance. B) Endoscopic view of sphenotomy after healing. Left side with septum (S) on left and inferior turbinate (IT) on right. Large sphenotomy (arrow) has been performed at top of sphenoethmoidal recess, above nasopharyngeal vault (NPV).

be controlled and the sphenoid sinus remained air-filled (Fig 2B).

DISCUSSION

We report 3 cases of sphenoidal mucocele following pituitary surgery diagnosed during an 18-month period in our institution. These observations suggest that the frequency of this disorder might be underestimated. The anatomic changes induced by surgery should not hinder this diagnosis, especially in the case of headache or visual complications long after surgery.

The physiopathology of mucocele remains the subject of ongoing debate.[5] However, it has become clear that the obstruction of the ostium of the paranasal sinus is a decisive step in the development of the disease. The latter may result from anatomic conditions or from surgery, either through intentional exclusion of a sinus without complete removal of its mucosa, or through changes in the surgical wound. As demonstrated by immunocytochemistry,[5] the persistence of the epithelial products in a closed cavity results in the production of cytokines such as interleukin-1 by the epithelial cells, which cause the erosion of bone overlying the expanding mucocele. Manifestations of sphenoidal mucocele include headache, decrease in visual acuity, diplopia, and exophthalmos.[6]

The technique of transsphenoidal pituitary surgery has been thought to require the removal of the sphenoid sinus mucosa in order to avoid any bacterial inoculum when opening the sella turcica. The sphenoid ostia are identified as landmarks for the removal of the sphenoid rostrum[7] before setting up the retractor, but enlargement of the ostia is mandatory to allow an adequate view of the pituitary fossa. The delayed development of a mucocele requires packing of the sphenoid sinus with fascia, muscle, or even fat after removal of the tumor — as well as incomplete removal of the sinus mucosa, when extensive sinus pneumatization does not allow control of the whole surface of the mucosa under the microscope. In the latter case, a right-angle endoscope might be useful in removing any epithelial remnant, especially when the pterygoid process is pneumatized. Recently, reports on conservative transsphenoidal pituitary surgery showed promising results with the use of endoscopy: they precluded removal of sphenoidal mucosa or packing of the sinus with nonabsorbable material.[8]

Diagnosis of an iatrogenic mucocele could often be made before clinical symptoms occur, since radiologic follow-up is usually performed over several years, particularly in the case of nonsecreting tumors. However, interpretation of the images is difficult because of disturbances related to surgical manipulation. Complete or partial opacification of the sphenoid sinus caused by blood, debris, implant material, or retention is found in virtually all patients on early postoperative imaging.[9] With time, the material within the sphenoid is resorbed in the majority of cases.[10] However, there are no precise data concerning late radiologic follow-up of large series of patients. The cases we report suggest that the existence of a sphenoidal mucocele should be questioned in case of recurrent headache after pituitary surgery — a complaint in up to 10% of patients after transsphenoidal hypophysectomy, according to Gammert.[11] When complete opacification of the sphenoid is present, the reinforcement of the lining of the sinus or the association of hypersignal on T1- and T2-weighted images is suggestive of a mucocele, although the signal characteristics of the inner fluid are highly variable, depending on the protein content.

The treatment consists of endoscopic transnasal marsupialization, since the mucocele, which may develop as a tumor, is in fact lined by a normal epithelium with an inflammatory reaction that will heal with drainage. This approach seems more reliable than total removal of the epithelial wall, which here might once again leave epithelial remnants sequestered from the nasal cavity and, further, allow cerebrospinal fluid leakage. At the time of surgery, prevention would require either endoscopic control of the mucosal remnants in case of sinus exclusion, or leaving the sphenoid sinus air-filled under a sealed sella.[8]

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RHINOLOGY SYMPOSIA

The 18th International Symposium on Infection and Allergy of the Nose (ISIAN), the 4th Asian Research Symposium on Rhinology (ARSR), and the 3rd ASEAN Rhinology Group Congress (ARC) will be held in Manila, Philippines, February 14-18, 1999. For information, contact Gil M. Vicente, MD, Congress President, Dept of ORL-HNS, Jose Reyes Memorial Medical Center, Rizal Avenue Sta. Cruz, Manila, Philippines 1003; telephone (632) 743-6921; fax (632) 732-1077 or (632) 522-0946; e-mail isian@psohns.com; web site http://www.psohns.com/isian.

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EFFECTS OF ANGIOGENIC GROWTH FACTORS AND A PENETRANCE ENHANCER ON COMPOSITE GRAFTS

DAVID B. HOM, MD MARGARET WINTERS, MD

MINNEAPOLIS, MINNESOTA

Skin-cartilage composite grafts are invaluable tissues used in facial reconstruction, yet their survival is unpredictable beyond a 1-cm diameter. In this study, the angiogenic growth factors basic fibroblast growth factor (bFGF) and endothelial cell growth factor (ECGF) and a penetrance enhancer (dimethyl sulfoxide [DMSO]) were applied to composite grafts to determine their effects on survival and vascularization. We applied ECGF, bFGF, and DMSO either topically or by intradermal injection to 120 auricular composite grafts (3.0 cm diameter) in New Zealand White rabbits. Dermabrasion was performed in 2 groups to attempt to increase transdermal delivery. Graft viability and vascularity were evaluated 3 weeks later by template analysis and angiography. In the results, ECGF and bFGF, when grouped together, had a 40% increase in vascular ingrowth as compared to controls ($p < .001$). However, neither ECGF nor bFGF increased graft survival. A coincidental finding was that DMSO with dermabrasion significantly improved graft viability (>100%) with or without an angiogenic agent ($p < .02$). The potential of DMSO with dermabrasion to increase composite graft viability warrants further investigation.

KEY WORDS — graft, graft survival, growth substance, vascularization.

INTRODUCTION

In facial reconstructive surgery, use of skin cartilage composite grafts has been limited by their critical size. Graft survival becomes unpredictable beyond a 1.0-cm diameter, and poor survival often results beyond this dimension.[1] Several studies have attempted to increase composite skin-cartilage graft survival. MacCollum and Grabb[2] reported increasing the survival of 1.5-cm composite grafts by deepithelializing the recipient bed 11 days before graft transfer. Hartman and Goode[3] describe the administration of intramuscular methylprednisolone to increase composite graft survival. Methylprednisolone was believed to minimize graft edema and perivascular inflammatory infiltrates, thus helping maximize blood flow to the graft. In several other studies, corticosteroids have been shown to be beneficial in increasing composite graft survival.[4,5]

With the advent of angiogenic growth factors, it may be possible to increase skin-cartilage composite graft survival. Angiogenic growth agents have been demonstrated to increase neovascularization in vitro and in vivo.[6] In animal models, angiogenic growth factors have been shown to increase neovascularization in skin flaps, resulting in increased flap viability.[7,8]

In particular, fibroblast growth factors (FGFs) have been promising in inducing neovascularization in vivo.[9] In microgram quantities, basic FGF (bFGF) induces endothelial cell mobilization and replication. Basic FGF is also a potent mitogen to fibroblasts and chondrocytes and can promote cartilage repair.[10] Hence, bFGF theoretically could be an agent to improve skin-cartilage composite graft vascularization, survival, and critical size. Another angiogenic growth factor in the FGF family is also very promising. Endothelial cell growth factor (ECGF) is a neural extract derived from bovine hypothalamus that is capable of stimulating neovascularization at 1,800 μg/mL in soft tissue.[8] One study testing an auricular amputation-reimplantation model did not show increased survival when bFGF was applied topically.[5] It appears that the method of delivery of FGF to the target tissue plays a very important role.

One very accessible and practical means to deliver a growth factor to composite grafts would be by a transcutaneous route. Sustained transcutaneous administration of nitroglycerin and scopolamine has been a well-established and effective means for systemic drug delivery. The stratum corneum is the major source of resistance to penetration of exogenous molecules across the skin.[11] With the application of a penetrance enhancer such as dimethyl sulfoxide

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From the Division of Facial Plastic and Reconstructive Surgery, Department of Otolaryngology–Head and Neck Surgery, University of Minnesota School of Medicine (Hom), and the Departments of Otolaryngology (Hom) and Radiation Oncology (Winters), Hennepin County Medical Center, Minneapolis, Minnesota. This project was funded by a research grant from Hennepin Faculty Associates and the Lions Multiple District 5M. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the Minneapolis Medical Research Foundation.

CORRESPONDENCE — David B. Hom, MD, Division of Facial Plastic and Reconstructive Surgery, Dept of Otolaryngology–Head and Neck Surgery, University of Minnesota School of Medicine, Box 396, UMHC, Minneapolis, MN 55455.

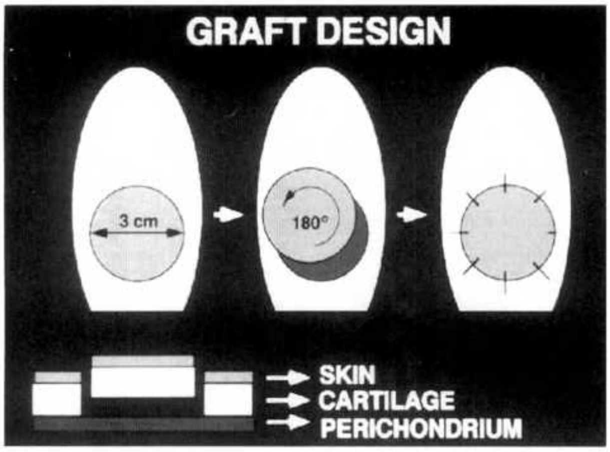

Fig 1. Composite skin-cartilage graft taken from dorsal aspect of auricle. After composite graft is harvested down to opposite perichondrium, graft is rotated 180° and sutured back.

(DMSO), small peptides such as insulin (6,000 d) and somatostatin can be administered transdermally and exert their effects.[12,13] The penetrance enhancers act by decreasing the diffusion barrier of the stratum corneum. It is assumed that detection of a transdermally administered drug in the systemic bloodstream indicates adequate absorption of the drug. However, little is known about the local effect of the drug in the dermis. Sodicoff et al[14] studied the transdermal administration of (^{14}C)cysteine in mice and demonstrated increased concentrations in the epidermis and dermis after pretreatment of skin with DMSO. Smaller molecules (less than 1,000 d) can pass quickly through the skin into the bloodstream without retention in the dermis. Larger hydrophilic molecules, such as bFGF (18,000 d), have extremely low skin permeability and may be able to be retained in the dermis. By investigating the use of a penetrance enhancer such as DMSO in conjunction with an angiogenic growth factor, a novel means to deliver an angiogenic growth factor to a critically needed area of vascularization in the dermis would be explored.

To our knowledge, the effect of angiogenic growth factors with a penetrance enhancer on composite graft survival has not been determined. Theoretically, if the neovascularization of composite grafts could be enhanced by angiogenic growth factors, their critical size and usefulness in facial reconstruction could be significantly increased. By enhancing composite graft survival, difficult-to-repair defects located in the nose, ear, and eyelid could be more easily reconstructed to achieve more optimal functional and structural results.

The aims of this study were 1) to determine if the angiogenic growth factors bFGF and ECGF can increase survival and vascularity of auricular skin-cartilage composite grafts, and 2) to evaluate if angiogenic growth factors may be effectively delivered transcutaneously to increase vascularity and viability of composite grafts by means of a penetrance enhancer (DMSO) and dermabrasion (DB).

MATERIALS AND METHODS

In this project, 2 angiogenic growth factors and 1 penetrance enhancer were tested. Endothelial cell growth factor, a neural extract derived from bovine hypothalamus capable of stimulating neovascularization at 1,800 µg/mL in soft tissue,[8] was obtained from Organon Teknika Corp Biotechnology Research Institute, Rockville, Md. Basic FGF, of which the recombinant human form can stimulate endothelial cell proliferation and mobilization in nanogram quantities,[9] was obtained from (formerly) Synergen, Boulder, Colo. Dimethyl sulfoxide, a penetrance enhancer and superoxide radical scavenger known to chelate iron, was obtained from Sigma, St Louis, Mo.

Animal Model. Sixty-five New Zealand White rabbits (3 to 4 kg) were used in this study, of which 60 animals survived the full protocol. In the rabbit auricle model, the ears are readily accessible for frequent inspection. Also, their auricles are large enough to design circular composite grafts of 7.1 cm^2 in size (3 cm diameter) on the dorsal surface. With a 7.1-cm^2 composite graft, the control grafts will demonstrate minimal viability (less than 40%), according to previous studies.[3]

The rabbits were sedated with xylazine hydrochloride (9 mg/kg), atropine sulfate (0.16 mg), and ketamine hydrochloride (40 mg/kg) intramuscularly. Chloramphenicol sodium 100 mg intramuscularly was administered preoperatively. The dorsal aspects of both ears were shaved and sterilely prepared with povidone-iodine solution. The auricular dorsoneurovascular bundle was then anesthetized with 0.5 mL of 1% lidocaine hydrochloride. A circular 3.0-cm-diameter template was used to trace the dimensions of the composite graft on the dorsal aspects of both ears. Similar to previous composite graft models, the graft was harvested to the level of the ventral perichondrium and rotated 180° and placed back in the wound[3] (Fig 1). The ventral perichondrium was left intact. The grafts were sutured in place with 6-0 nylon suture in a running fashion. This was performed on both ears.

The completed study consisted of 6 animal groups (10 rabbits per group, 20 grafts per group; Fig 2). In group 1 (bFGF topical delivery), bFGF in a phosphate buffered saline (PBS) solution at 10 mg/mL (a total of 75 µg for each 7.1-cm^2 composite graft) was applied *topically* to the wound bed prior to placing and suturing the graft into place. Control grafts were

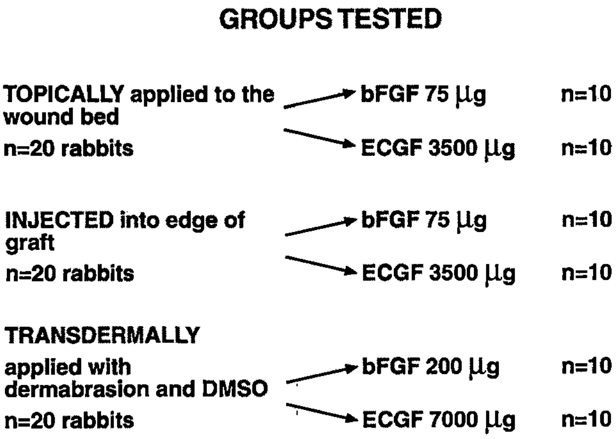

Fig 2. Distribution of tested groups by delivery (topical, injection, or transdermal) and growth factor (basic fibroblast growth factor [bFGF] or endothelial cell growth factor [ECGF]). DMSO — dimethyl sulfoxide.

done in an identical fashion with an equal volume of PBS solution.

In group 2 (bFGF delivery by injection), bFGF in a PBS solution at 300 µg/mL was *injected* subcutaneously into 12 quadrants (6 µg per quadrant, 0.02 mL) with a tuberculin syringe into the edge of the composite graft (75 µg per graft). Composite grafts were then sutured into place. Controls were performed in an identical fashion with an equal volume of PBS solution.

In group 3 (ECGF topical delivery), ECGF at 10 mg/mL in PBS solution (total 3,500 µg for each 7.1-cm^2 composite graft) was applied *topically* to the wound bed before placing the graft and suturing it into place. The control was done in an identical manner with an equal volume of PBS solution.

In group 4 (ECGF delivery by injection), ECGF (3,500 µg) was *injected* subcutaneously into 12 quadrants of the edge of the composite graft (290 µg per quadrant). The composite grafts were then sutured into place. The control was done in an identical fashion with an equal volume of PBS solution.

In group 5 (bFGF delivery with DMSO and DB), on the planned graft donor site, light DB down to the superficial papillary layer was performed with a diamond fraise to abrade the major barrier of the skin, the stratum corneum. The composite graft was then harvested. The graft skin and skin of the recipient edge were pretreated with 200 µL of 100% DMSO (Sigma) for 15 minutes. The grafts were sutured back into place. Gelfoam soaked with 200 µg of bFGF was placed over the graft, and an occlusive dressing (Tegaderm, 3M, St Paul, Minn) was applied. The dressing and bFGF-soaked Gelfoam were then removed after 48 hours. The control ear had Gelfoam soaked in an equal volume of PBS solution placed over the DMSO-pretreated graft in a similar fashion.

In group 6 (ECGF delivery with DMSO and DB), on the graft donor site, light DB was performed down to the superficial papillary layer of the dermis with a diamond fraise to remove the stratum corneum. The composite graft was harvested in a similar manner, and the skin of the recipient edge was pretreated with 200 µL of 100% DMSO (Sigma) for 15 minutes. The graft was sutured into place. Gelfoam soaked with 7,000 µg of ECGF was placed over the graft, and an occlusive dressing (Tegaderm) was applied. The dressing and ECGF-soaked Gelfoam were then removed after 48 hours. The control ear used Gelfoam soaked in an equal volume of PBS solution placed over the DMSO-pretreated graft in a similar fashion.

Viability Assessment. Twenty-one days after surgery, templates were traced of the entire graft and of the clinically viable areas of the graft. These templates were analyzed by Zeiss Interactive Digital Analysis System (ZIDAS, Minneapolis, Minn) polar planimetry to quantitate the viable skin area remaining on each graft as percent viability.

Angiographic Preparation. After template analysis, angiography of the grafts was performed on day 21 to determine the extent of neovascularization of the treated and control composite grafts. The rabbits were anesthetized with xylazine hydrochloride (9 mg/kg), atropine sulfate (0.16 mg), and ketamine hydrochloride (40 mg/kg) intramuscularly. Both common carotid arteries were exposed, proximally ligated, and cannulated with a 22-gauge catheter. Porcine heparin (1 mg/kg) was infused through the catheter. After 5 minutes, an infusion of 30% micropaque in normal saline was started at a constant pressure of 140 mm Hg. The animal was then painlessly sacrificed with pentobarbital sodium 15 mg/kg and exsanguinated through a femoral arteriotomy. The micropaque infusion was continued until the draining blood whitened (approximately 800 mL of micropaque solution). Then the micropaque solution (30% in 10% formalin) was infused until muscle fasciculations were seen (approximately 400 mL of solution). The rabbit ear bases were clamped, severed, and fixed in 10% formalin for 48 hours. The composite grafts were peeled from the ear, and by mammography techniques, angiograms of the grafts were performed to detect the degree of graft vascularity (Fig 3). The technique of the mammography settings was at a magnification of 1.7, with 23 kilovoltage peak at 20 mA/s. A 0.1-mm focal spot was used with Kodak MinR film in a 90-second processor on a single emulsion screen.

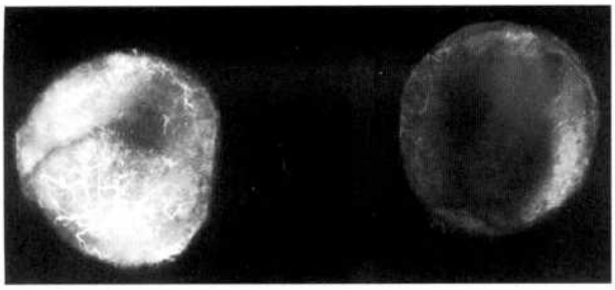

Fig 3. Angiograms of composite skin-cartilage grafts. Left graft in photo is ECGF topical–treated graft. Right graft in photo is control graft.

The resultant angiograms were then analyzed to *quantitate* the contrast present in the perfused vessels of the composite graft by using an image processor at the Biomedical Image Processing Laboratory (BIPL, University of Minnesota Computer Laboratory Facility). All grafts were analyzed with identical parameter settings in order to obtain standardization of the measurements.

In addition to comparing the vascular and viability differences between the treated and control grafts within each group, comparisons were made with the following groups: DMSO with the angiogenic growth factors (groups 5 and 6); and those treated topically (groups 1 and 3) and by intradermal injection (groups 2 and 4).

Further, the control grafts of groups 1 through 4 were compared to the control grafts of groups 5 and 6 to determine whether DMSO and DB, by themselves, had a direct effect on graft viability and neovascularization. The results were evaluated by analysis of variance (ANOVA) to determine the significance of the differences between the test groups.

RESULTS

Sixty of 65 rabbits survived the length of the study. Of the 5 rabbits that did not survive the length of the study, 2 had a failure to thrive, 1 did not tolerate anesthetic injection, 1 developed a cheek abscess, and 1 excessively traumatized both ear grafts. In all animals, the ischemic areas of the skin that did not survive became necrotic over 14 days.

Vascularization Results. Composite graft vascularization after 21 days in each group is described in Table 1 (mean vascular units ± SE). By ANOVA, both ECGF and bFGF grouped together (N = 60) statistically increased vascular ingrowth (mean 7.5 units) compared to controls (mean 5.4 units), with the standard error of this difference being 0.55 ($p < .001$). Each growth factor, ECGF and bFGF, appeared equally effective in increasing vascular penetration, and it did not matter what the method of delivery was (topical, by injection, or with DB and DMSO). However, in looking at each group individually (N = 10), there was no statistical significance, due to the decreased numbers in each group.

When comparing the mean vascularity difference between the DMSO-DB without growth factor group and non–growth factor controls, there was a statistically significant difference of improved vascularity in the DMSO-DB group (mean 6.88 versus 4.69, respectively; $p < .011$).

Viability Results. With viability, the mean viabilities ± SE are given in Table 2. When the angiogenic growth factor groups (bFGF and ECGF) were combined and compared versus their controls, the mean viabilities were 11.15 and 10.4, respectively, approaching a statistically significant difference ($p = .548$), with the SE of the difference at 1.22.

There was a statistically significant difference in viability between each growth factor (bFGF and ECGF), but not when each was compared to the controls. This was because the bFGF application had a negative effect on composite graft viability, while ECGF had a positive effect. For ECGF, the mean viability was 13.68, versus 8.61 for the bFGF group. The effect of ECGF in increasing viability was influenced by the modality of its delivery. When ECGF was given topically or with DB and DMSO, a trend for increased viability was apparent.

The DMSO with DB significantly improved graft viability both with (mean 19.78 ± 2.89) and without (mean 19.35 ± 3.14) an angiogenic growth factor compared to the control groups without growth factor (mean by injection 6.38, mean by topical 5.48), with $p < .001$ for both.

DISCUSSION

In this study, the angiogenic factors bFGF and ECGF were delivered by several modalities (topically, by injection, with DMSO-DB) to attempt to

TABLE 1. COMPOSITE GRAFT VASCULARITY

Delivery	*bFGF Control*	*bFGF Treated*	*ECGF Control*	*ECGF Treated*
Topical	4.31 ± 0.77	5.14 ± 1.34	6.02 ± 1.07	8.15 ± 0.98
Injection	3.21 ± 0.89	5.77 ± 1.34	5.22 ± 0.71	7.23 ± 1.36
Transdermal	7.68 ± 1.23	11.20 ± 2.01	6.08 ± 0.86	7.27 ± 0.91

Data are vascular units, mean ± SE.

bFGF — basic fibroblast growth factor, ECGF — endothelial cell growth factor.

TABLE 2. COMPOSITE GRAFT VIABILITY

Delivery	*bFGF Control*	*bFGF Treated*	*ECGF Control*	*ECGF Treated*
Topical	3.81 ± 1.69	0.92 ± 0.63	7.15 ± 2.56	11.12 ± 2.90
Injection	4.92 ± 2.08	7.70 ± 2.79	7.83 ± 3.25	7.59 ± 3.00
Transdermal	23.07 ± 4.64	17.21 ± 2.98	15.62 ± 4.13	22.34 ± 4.98

Data are percent viability, mean ± SE.

increase viability and vascularity of skin-cartilage composite grafts. The DMSO and DB were used to attempt to increase transdermal penetration of the angiogenic growth factor into composite skin-cartilage grafts. The angiogenic growth factors did increase graft vascularity; however, they did not appear to increase viability. In fact, the DMSO and DB together appeared to increase vascularity and viability most significantly.

The survival of skin-cartilage composite grafts depends on a number of factors, similar to that of full thickness skin grafts: plasmatic inhibition during the first 24 to 48 hours, followed by neovascularization, by inosculation, and by direct vessel ingrowth 2 days after surgery.[15] It is believed that the cartilage in composite grafts may act as a mechanical barrier limiting vascularization.[16-18] Vascularization is thought to be the key mechanism for long-term composite graft survival. During the revascularization of a skin-cartilage composite graft, the host bed vessels are unable to penetrate cartilage. The host vessels propagate along the underside of the cartilage until the graft dermis is reached. At this location of the graft, vascularization occurs in the dermis portion of the graft.[19] Previous stereoscopic studies have demonstrated that vascular flow in composite grafts begins after 48 hours.[20] Initially, blood flow is seen at the graft-host dermal junction. Then, over several days, blood flow is seen toward the center of the graft perichondrium.

Surgical techniques to increase vascularity to composite grafts include increasing the recipient host bed exposure to the graft, and using more skin in proportion to cartilage in the composite graft. One study showed that composite ear graft survival can be enhanced by surgically transposing an adjacent vascular pedicle to the underside of the composite graft to increase perivascular angiogenesis.[15] The survival of composite skin-cartilage grafts is similar to that of skin grafts, in that dermal revascularization is critical for long-term viability.

The cornified layer of the stratum corneum of the epidermis is the major barrier to transcutaneous drug penetration.[21] Penetrance enhancers are compounds that are added to vehicles to alter the molecular environment of the epidermis to facilitate drug absorption. In this study, DMSO was used as a penetrance enhancer to attempt to increase transcutaneous delivery of angiogenic growth factors. In addition, DB was performed to mechanically remove the stratum corneum layer down to the superficial papillary dermis, to allow more growth factor to traverse the dermis.

A coincidental finding in this study was that the combination of DB and DMSO with or without the growth factors improved graft viability ($p < .001$) and vascularity ($p <. 011$) compared to all control groups. Unfortunately, DB and DMSO were not tested separately in this study to determine their individual effects on composite grafts. To accomplish this, additional groups testing DB alone and DMSO alone would be required. This was not performed in this study because the original objective of this project was to investigate the effects of bFGF and ECGF on composite grafts with different delivery modalities. To determine if the growth factors traversed the dermis in the penetrance enhancer group, radiolabeling of the growth factors would be helpful in the future in detecting their presence in the systemic circulation.

In this study, DMSO, in addition to being a penetrance enhancer, appeared to behave also like a free radical scavenger. This was because when DMSO was used, an increased viability was seen even without the angiogenic agents. In a study by Aden and Biel,[4] DMSO decreased necrosis of skin-cartilage composite grafts by 27% when given preoperatively (40 mg/kg intramuscularly). They believed the beneficial mechanism of DMSO was from its iron-chelating effect that prevents ferrous-dependent lipid peroxidation, thus minimizing oxygen radical formation.[4] This mechanism was apparent especially in the ECGF group, from the fact that even though there was no increased vascularity with DMSO controls, increased viability was evident.

Further, DMSO has the ability to scavenge for free radicals. In a rodent study, 10% DMSO and 20% DMSO were thought to protect against doxorubicin-induced tissue necrosis by this mechanism.[22] Specifically, DMSO is considered a hydroxyl free radical scavenger, and its metabolic component, dimethyl sulfide, is an oxygen free radical scavenger. When applied to doxorubicin soft tissue extravasation sites, topical DMSO decreased ulcer size.[22]

Previous studies have investigated bFGF at lower doses in an auricular reimplantation model in the rabbit. In a study by Henrich et al,[5] when bFGF was given at a smaller dose (20 ng/mL) topically over a 5-day course, no increased survival was seen. However, when corticosteroids were given, they found that the survival increased. The mechanism for this finding appeared to be increased neovascularization.[5] In a study by Arden et al,[15] the angiogenic factor lipid omental extract, injected parenterally, was not successful in increasing chondrocutaneous graft survival following vessel implantation.

In our study, increased composite graft viability with the angiogenic growth factors bFGF and ECGF was not seen for several possible reasons. The increased amount of vascularity present may not have been sufficient to increase viability. When all of the groups treated with the angiogenic growth factors were grouped together and compared to their controls, a statistically increased vascularity was seen. However, a threshold of vascularity may be required in order to increase composite graft viability.

In attempting to deliver the angiogenic growth factor transdermally, with DMSO and DB, increased vascularity was seen with both growth factors; however, composite graft viability only increased with ECGF. It may be that bFGF became more unstable or could not cross the dermis. Other reasons may be that an inadequate concentration was available or the timing of delivery was not optimal.

In conclusion, ECGF and bFGF, when delivered topically, by injection, or with a penetrance enhancer, did not increase survival of skin-cartilage composite grafts. An interesting finding was that DMSO with DB has the potential to increase composite graft viability. In the future, the action of DMSO in relation to DB needs to be further explored in composite grafts.

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THIRTY-FIVE–MILLIMETER PHOTOGRAPHY USING THE KANTOR-BERCI VIDEO LARYNGOSCOPE

BRUCE BENJAMIN, FRACS, FAAP

SYDNEY, AUSTRALIA

The Kantor-Berci model II laryngoscopes employ a centrally located rigid telescope. Although the primary application is for video laryngoscopy, the system can also be used for 35-mm photography during microlaryngeal operations. The fixed, unchangeable field of view and the great depth of focus make this system ideal for photographic documentation during endolaryngeal surgery without interruption of the procedure.

KEY WORDS — Kantor-Berci laryngoscope, laryngeal photography.

INTRODUCTION

Since Kleinsasser[1] first reported use of the operating microscope in 1963, techniques for endolaryngeal diagnosis and microsurgery have included 35-mm photography and video recording for documentation.

In 1991 Kantor et al[2] introduced a new approach to microlaryngeal surgery for adult patients using a laryngoscope with a channel on the left side for a rigid telescope to which a small video camera was attached (Fig 1A). This system was advocated not only for diagnostic examination, but also for endolaryngeal surgery performed when viewing the image on a television screen; however, the technique has not been widely adopted, as most surgeons prefer to operate using the microscope.

Yanagisawa et al[3] compared the Kantor-Berci telescopic technique to the standard technique with an operating microscope. They found problems with the large size and the design of the Kantor-Berci laryngoscope, which was awkward to introduce in many patients. However, in patients in whom the laryngoscope could be satisfactorily positioned, the telescopic image provided great depth of field and good visualization of the anterior glottis, but a less complete view of the posterior glottis. Manipulation of microsurgical instruments was often difficult, particularly for right-sided laryngeal lesions, because the left-sided position of the telescopic housing, at the proximal end of the laryngoscope, got in the way. They concluded that the technique was an excellent method for video documentation and for teaching.

These difficulties with the original laryngoscope have been corrected by introduction of the new Kan-

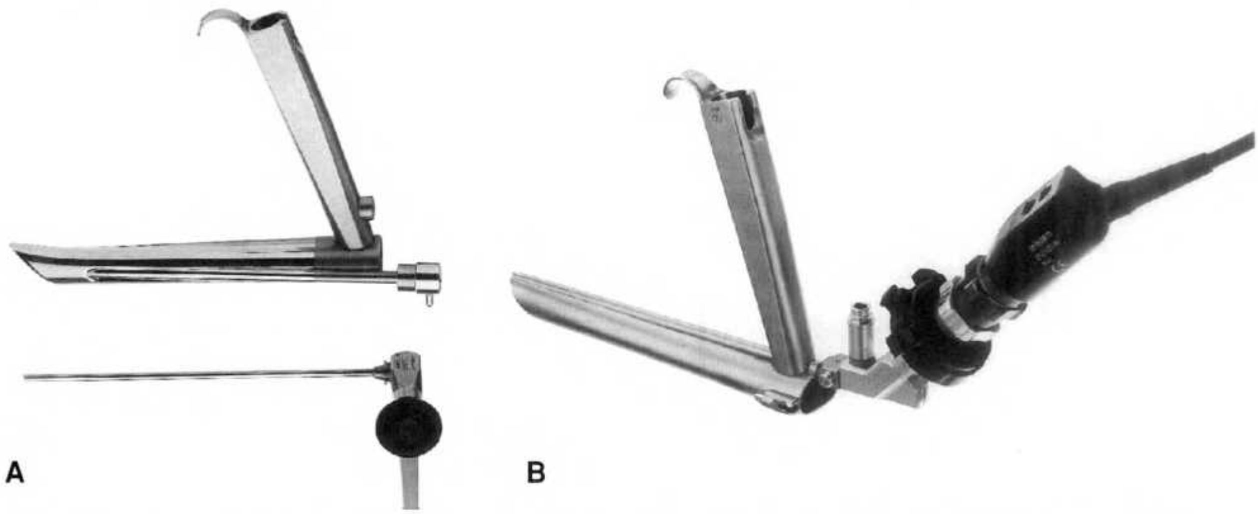

Fig 1. A) Original Kantor-Berci (model I) video laryngoscope. Left-hand–side channel for special telescope is offset at proximal end. B) New, improved Kantor-Berci (model II) video laryngoscope (larger size). Telescope is located in superior central channel. Telescope is 4.0 mm in diameter and has 15° angle view. Video camera is attached.

From the Department of Otolaryngology, Royal North Shore Hospital, St Leonards, Australia. Supported by Karl Storz GmbH and Co, Tuttlingen, Germany.

CORRESPONDENCE — Bruce Benjamin, FRACS, FAAP, 231 Macquarie St, Sydney, NSW 2000, Australia.

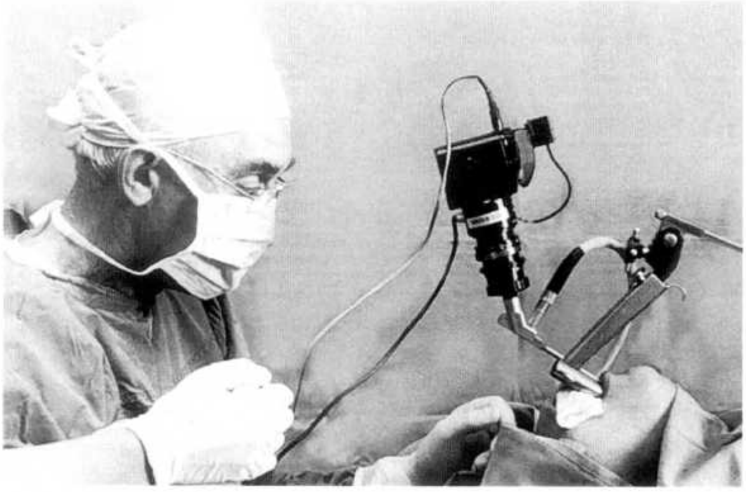

Fig 2. 35-mm camera (with shutter release cable) is attached to telescope.

tor-Berci model II videolaryngoscopy system (Fig 1B), which offers a choice of two adult laryngoscopes — one of large diameter (Karl Storz 8590CV) and one of small diameter (Karl Storz 8590SV) — both of which give very good exposure of the glottis. At the superior aspect of each laryngoscope there is a centrally located, recessed channel and a proximal housing that secures the special 17-cm straight-forward 15° telescope (Karl Storz 8575AV) in place, leaving an unobstructed lumen in the laryngoscope for manipulation of microsurgical instruments.

This laryngoscopy system is advantageous for documentation of endolaryngeal surgical procedures because the telescope gives a fixed, unchangeable field of view. For demonstration and teaching purposes, 35-mm photographs can be taken to illustrate steps in various surgical procedures. The author's technique of laryngeal photography using rigid telescopes has been previously described in detail.[4]

METHOD

Under general anesthesia, one of the two laryngoscopes, with lighting by a Benjamin-Havas clip, is positioned and manipulated to give optimal visualization and is then placed in suspension. The telescope, whose distal end has been warmed or dipped in antifog solution, is passed down the central telescope channel and locked into position, and a light cable from the photographic unit is connected. The 35-mm camera is positioned and held in place by the spring-loaded adaptor on the front of its zoom lens (Fig 2). The presence of the telescope and camera does not interfere with diagnostic examination, microsurgery, or laser surgery, all of which can be performed in the usual way, using a microscope.

A shutter release cable (Fig 2), to prevent camera shake, is activated by an assistant when requested by the surgeon. If the weight of the 35-mm camera makes the assembly seem unstable, it should be steadied by an assistant.

RESULTS

Examples of composite photographs made by this method are shown in Fig 3.

DISCUSSION

The primary application of the Kantor-Berci system is for video laryngoscopy, but it can be used for 35-mm photography of the glottis. Laryngeal surgery can be performed with the naked eye or with a microscope, and a series of photographs can be taken without interruption of the procedure. After processing the film, a composite photograph representing each step is made.

The design of the improved model II laryngoscopes does not allow photography of the posterior glottis, and exposure of the supraglottis, subglottis, and posterior glottis is usually unsatisfactory. There are no laryngoscopes for pediatric laryngoscopy. Nevertheless, many procedures can be photographed, including biopsy methods; removal of cysts, nodules, or papillomas; injection of Gelfoam or Teflon paste; laser treatment of vocal granuloma, dysplasia, or early invasive carcinoma; dissection of an intracordal cyst; and other operations on the membranous vocal folds.

The Kantor-Berci system has a number of advantages for 35-mm still photography. First, the telescope is fixed and the field of view remains the same in every photograph. Second, the extended depth of focus, as with all Hopkins telescopes, maintains the instruments and the lesion in focus — a distinct advantage when compared to photographs taken through a beam splitter on an operating microscope. Third, because the camera and telescope remain "separate" from the laryngoscope lumen, photography does not interrupt the endoscopic procedure.

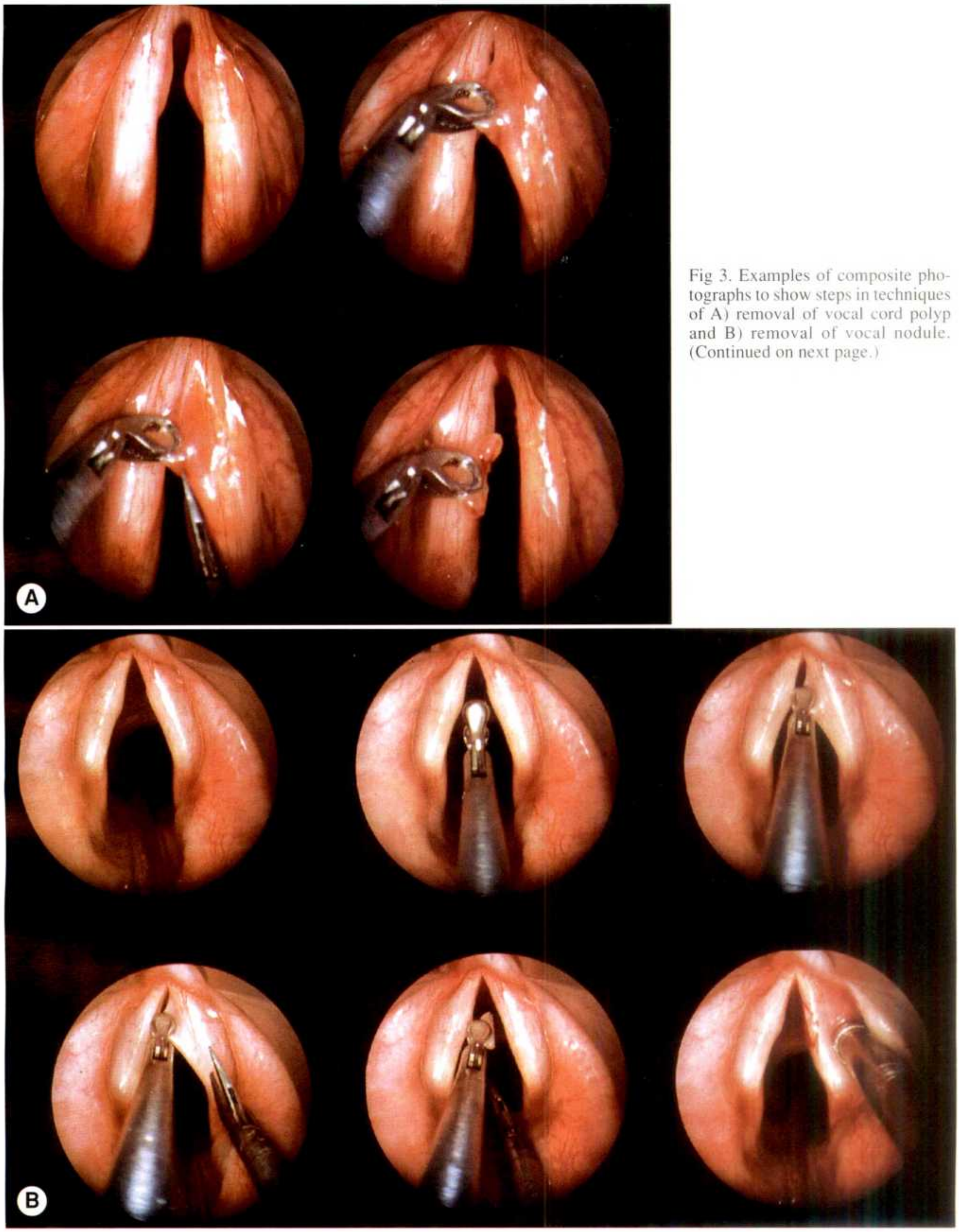

Fig 3. Examples of composite photographs to show steps in techniques of A) removal of vocal cord polyp and B) removal of vocal nodule. (Continued on next page.)

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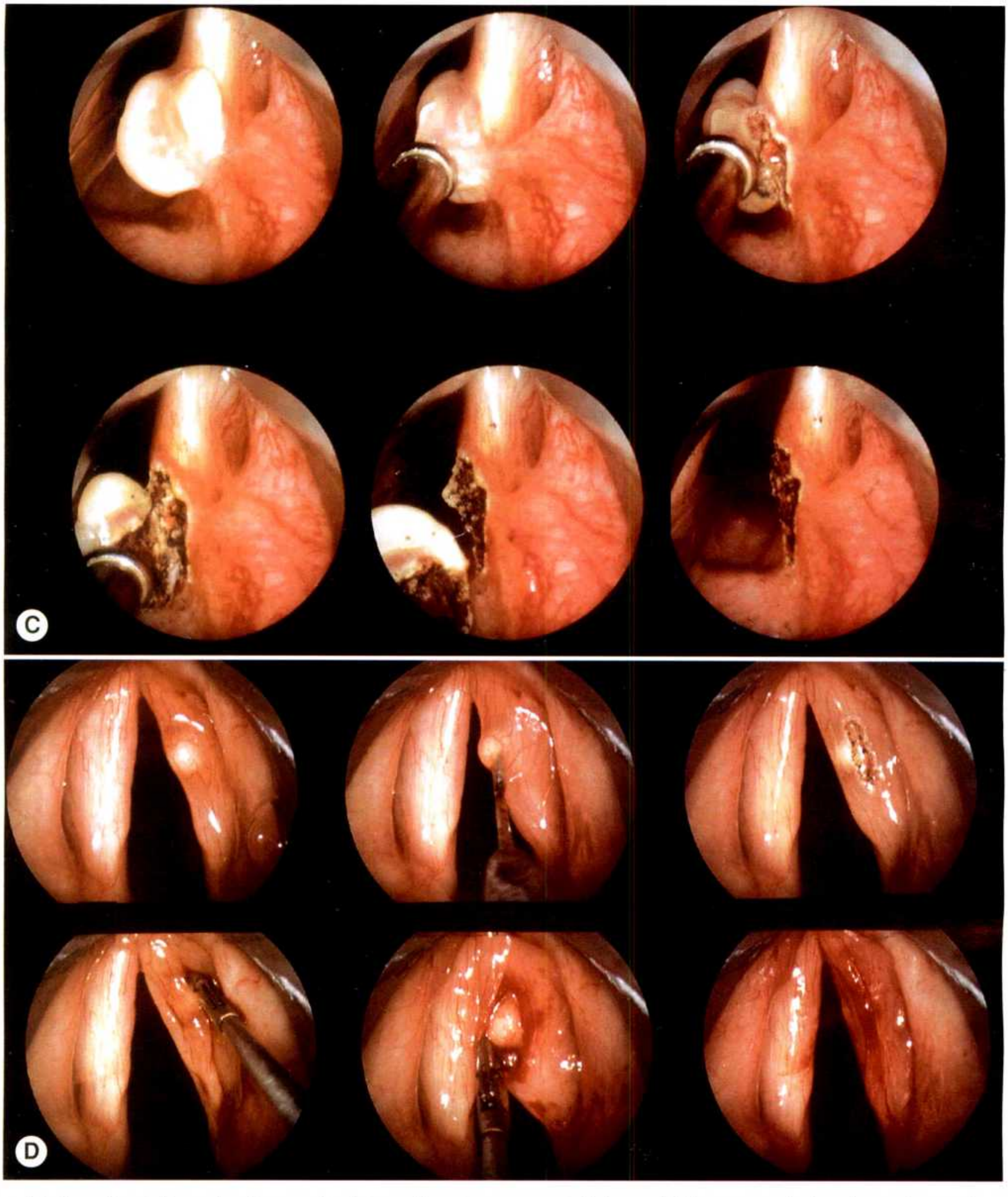

Fig 3, continued. Examples of composite photographs to show steps in techniques of C) laser removal of vocal granuloma and D) dissection removal of intracordal cyst.

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ELECTROPORATION THERAPY OF HEAD AND NECK CANCER

WILLIAM R. PANJE, MD
CHICAGO, ILLINOIS

MICHAEL P. HIER, MD
MONTREAL, CANADA

GUY R. GARMAN, DO
MARYVILLE, TENNESSEE

ERIN HARRELL, BSN, RN
CHICAGO, ILLINOIS

ANDREW GOLDMAN, MD
NEW YORK, NEW YORK

ISAC BLOCH, MD
FLORAL PARK, NEW YORK

The purpose of this paper is to introduce the concept of electroporation therapy and present our results from using this new technique combined with intralesional bleomycin in head and neck cancer patients. Electroporation therapy is a technique wherein high-voltage electric impulses delivered into a neoplasm transiently increase cell membrane permeability to large molecules, including cytotoxic agents. In this phase I/II study, extremely low-dose bleomycin sulfate was electroporated into head and neck malignant neoplasms in 10 patients. Tumor responses included 2 nonresponders, 3 partial responders, and 5 complete responders, with a mean follow-up of 40 weeks. We conclude that this technique offers promising possibilities in the local treatment of head and neck cancer.

KEY WORDS — bleomycin, electroporation, head and neck cancer.

INTRODUCTION

Conventional modalities used in the treatment of head and neck cancer include surgery, radiotherapy, and chemotherapy. A major limitation to all of these treatments is the morbidity inflicted, both locally and systemically. The technique of electroporation is a commonly used method for transfecting cells in the laboratory. It results in a reversible permeabilization of cell membranes induced by a square wave electrical impulse. The process of electroporation allows for drug entry into a cell's cytosol in very high concentrations.[1] Electroporation therapy (EPT) involves the combination of intralesional administration of a chemotherapeutic agent with the technique of electroporation.

Extensive animal research has been conducted that supports the notion that EPT augments the effectiveness of a chemotherapeutic agent. Response rates ranging between 70% and 100% have been achieved in various studies involving mice and rats, treating melanoma, hepatocellular carcinoma, lung carcinoma, breast carcinoma, sarcomas, and gliomas.[2-13] Bleomycin sulfate was found to concentrate within the tumor cell cytosol 700-fold after external application of the cytotoxic agent and EPT.[11]

Clinical EPT trials have been conducted by Mir et al[14] in metastatic head and neck cancer patients. They reported a 50% complete response rate to electroporation and systemic bleomycin in 28 treated dermal metastases.[14]

Clinical trials of electrochemotherapy to treat cutaneous and subcutaneous malignancies were initiated in 1995 at the University of South Florida (the Moffitt Cancer Center and the Department of Surgery). These trials were based on preclinical data.[15] Three EPT protocols were tested, involving 30 patients in all. The study included patients with melanoma, basal cell carcinoma, squamous cell carcinoma, adenocarcinoma, and Kaposi's sarcoma, and an approximate 95% complete response rate was seen with all neoplasms treated with electroporation and intralesional bleomycin.[16-18]

We report our experience in 10 patients with head and neck cancer (8 squamous cell carcinomas, 1 adenoid cystic carcinoma, and 1 adenocarcinoma) treated with EPT and low-dose intralesional bleomycin.

PATIENTS AND METHODS

Between August 1996 and May 1997, 10 patients with head and neck malignancies were treated with EPT and intralesional bleomycin (EPT[B]). The study group included 6 men and 4 women, ranging in age from 37 to 95.

Patient inclusion criteria were that patients had biopsy-proven head and neck cancer and were unable or unwilling to undergo surgery or radiotherapy, or that they had recurrent cancer and had failed prior radiotherapy and/or surgery.

All patients were fully informed about the investigational nature of the procedure. They all signed informed consents. The EPT(B) protocol was reviewed and approved by the Rush Presbyterian–St Luke's Medical Internal Review Board before commencement of the study. The research part of the project

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From the Department of Otolaryngology, Rush Presbyterian–St Luke's Medical Center, Chicago, Illinois.

CORRESPONDENCE — William R. Panje, MD, Dept of Otolaryngology, Rush Presbyterian–St Luke's Medical Center, 1725 W Harrison St, Suite 340, Chicago, IL 60612.

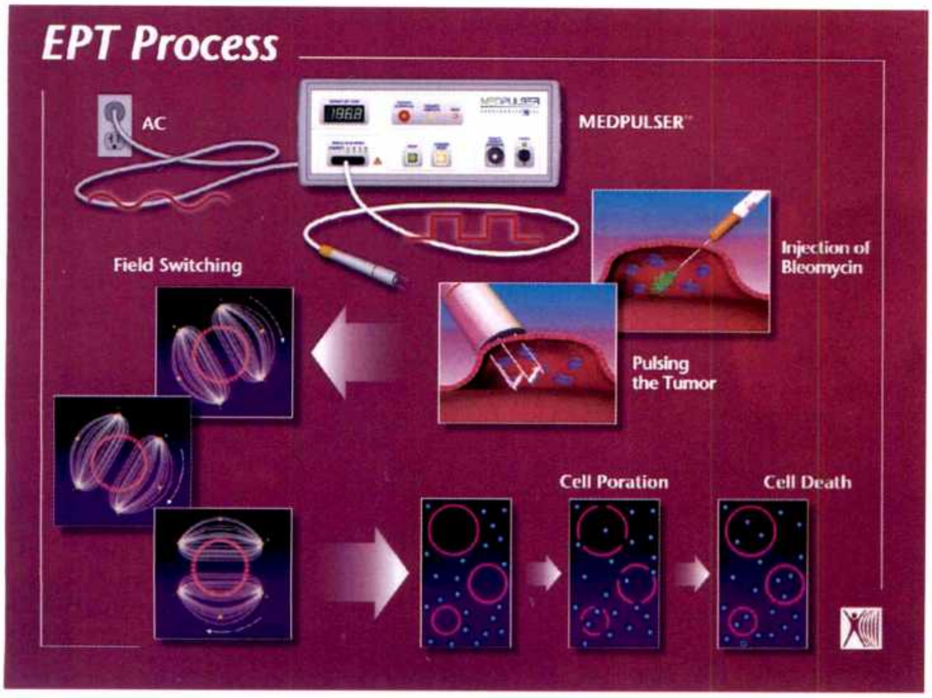

Fig 1. Electroporation therapy (EPT) process utilizing intratumoral bleomycin. Sinusoidal electrical energy is transformed into square wave energy pattern, which, when applied to previously bleomycin-injected tumor, produces cell wall pores with subsequent cytotoxic agent diffusion into cytoplasm and eventual neoplastic cell death.

was funded by Genetronics, Inc (San Diego, Calif). All of the procedures were administered in an outpatient setting or with a single overnight hospital stay. Patient 2 was an exception and required a prolonged hospital stay due to coexisting medical problems. The treatments were all conducted under general anesthesia, except for patient 7, who was treated under local anesthesia.

The tumor site was injected around its perimeter with 1% lidocaine hydrochloride containing 1: 100,000 epinephrine, in order to achieve vasoconstriction. Tumor volume was calculated from the following formula: $(A \times B \times C \times 3.14)/6$ = tumor volume (cm^3) where A = length of the longest diameter (cm), B = the next-longest diameter perpendicular to A (cm), and C = the maximum thickness or tumor depth (cm). The in vivo tumor dimensions were determined by bimanual palpation and/or magnetic resonance imaging or computed tomography scans prior to treatment.

Ten minutes following the epinephrine infiltration, the tumor was palpated or visualized and uniformly injected with bleomycin sulfate (Bristol-Myers Squibb, Princeton, NJ) at a dosage of 1 unit/cm^3 of tumor (Fig 1). The bleomycin was formulated by the Rush pharmacy in 1-mL tuberculin syringes in a 4-unit/mL solution. Twenty-five–gauge $1^{1}/_{2}$-in needles were used for tumor infiltration. Some tumors received 3 to 5 units/cm^3 because of extravasation during tumor injection.

The EPT pulse generators used were the Electrosquare Porator, 820 RN, and the Med Pulser (Genetronics, Inc), used in conjunction with a plastic rod tipped with a 6-needle electrode array forming a circle. The diameter of the electrode probe was 1 of 3 sizes: 0.5 cm, 1.0 cm, or 1.5 cm, with a needle length of 1 or 2 cm. Tumors larger than the electrode probe were encompassed by repeated insertions overlapping the previous insertion site by 3 to 5 mm. Standard voltage settings are predetermined for each of the 3 electrode arrays: 0.56 kV for the 0.5-cm probe, 1.13 kV for the 1.0-cm probe, and 1.69 kV for the 1.5-cm probe.[19]

After injection of bleomycin, approximately 1 to 3 minutes elapsed before electrode insertion into the tumor. The electrical pulses were then administered. The pulse length was 99 microseconds, with 6 pulses applied with each cycle. The number of cycles applied to each tumor ranged from 1 to 15, depending on tumor size. No more than 5 cm^3 was treated at one time. The largest tumor treated was 25.12 cm^3.

The patients were followed in the outpatient clinic weekly, and careful examination of the tumor was performed. One month post-treatment, multiple biopsy specimens were taken from the treatment sites and a second EPT treatment was administered if any of the biopsies were positive for cancer.

RESULTS

Results are presented in the Table.

Patient Response. Ten patients were treated over a period of 9 months. Eight squamous cell carcinomas, 1 adenoid cystic carcinoma, and 1 adenocarci-

PATIENT INFORMATION AND OUTCOMES OF ELECTROPORATION THERAPY

Patient	*Age*	*Sex*	*Tumor Type*	*Tumor Site*	*TNM Classification**	*Prior Treatment*	*EPT Date(s)*	*Outcome*
1	81	M	Squamous cell carcinoma	Nasal septum	rT2N0M0	Surgery and 6,500 cGy in 1991	Aug 14, 1996	Biopsy-proven complete response Oct 22, 1996
2	88	F	Squamous cell carcinoma	Retromolar trigone	rT4N0M0	7,000 cGy in 1995	Aug 30, 1996, and Sept 25, 1996	75% Partial response after 5 weeks; death from pneumonia Oct 24, 1996
3	75	F	Squamous cell carcinoma	Floor of mouth	yT2N0M0	7,000 cGy in 1996	Oct 2, 1996	Biopsy-proven complete response Jan 1997; death from metastases March 18, 1997
4	63	F	Squamous cell carcinoma	Floor of mouth	T2N0M1	Refused surgery and radiotherapy	Oct 4, 1996, and Nov 13, 1996	Lobectomy Dec 1996 for lung metastasis; biopsy-proven complete response March 1997
5	65	M	Squamous cell carcinoma	Oropharynx	yT4N0M0	6,850 cGy in 1996	Nov 18, 1996, and Dec 18, 1996	20% Local clinical response
6	95	M	Adenocarcinoma	Ethmoid sinus	rT4N0M0	6,500 cGy in 1996	Dec 9, 1996, and March 5, 1997	50% Clinical response March 1997
7	37	M	Squamous cell carcinoma	Maxillary sinus	rTxN3M?	Surgery and 6,500 cGy in 1996	Treated neck metastases Dec 11, 1996	10% Local clinical response; death from brain metastases Jan 12, 1997
8	76	M	Squamous cell carcinoma	Base of tongue	T2N0M0†	Refused surgery and radiotherapy†	Dec 11, 1996, and Jan 12, 1997	>50% Clinical response Feb 1997
9	53	F	Adenoid cystic carcinoma	Parotid	rT4N2M1	Neutron beam radiotherapy and chemotherapy	Dec 18, 1996, and March 3, 1997	Magnetic resonance–proven complete response April 1997
10	68	M	Squamous cell carcinoma	Supraglottic larynx	yT1N0M0	Combined chemotherapy and radiotherapy	May 28, 1997	Biopsy-proven complete response July 30, 1997

*Beahrs OH, Henson DE, Hutter RVP, Kennedy BJ, eds. American Joint Committee on Cancer. Manual for staging of cancer. 4th ed. Philadelphia, Pa: JB Lippincott, 1992 (y — persistent disease; r — recurrent disease).

†Patient had prior radiotherapy and partial glossectomy with neck dissection for contralateral adenocarcinoma of submaxillary gland in 1992.

noma were administered intralesional bleomycin, followed by 1 to 14 overlapping EPT(B) applications, sufficient to treat all of the tumor volume in 8 patients and partial volume in 2 patients. Five (50%) patients demonstrated complete responses, which is defined as no measurable or biopsy evidence of remaining cancer. Three (30%) achieved partial responses, which is defined as a >50% reduction in tumor volume. Two (20%) demonstrated an "insignificant" effect, which is defined as a <50% reduction in tumor volume. An overall 80% tumor response rate was achieved with EPT(B) treatment of head and neck cancers.

All patients demonstrated transitory masticator and tongue muscle spasm at the time of electroporation administration. In patient 7, who was treated under just local anesthesia, the muscle spasms were not painful, but definitely alarming to the patient. He failed to return for a second treatment because of cancer progression.

All of the other patients tolerated the treatments extremely well. Duration of general anesthesia was always less than 1 hour, averaging approximately 30 minutes. No significant side effects were attributable to the treatment itself. Most patients reported a reduction in pain compared to pretreatment at 1 to 2 weeks following EPT.

None of the patients demonstrated bleomycin toxicity. Even the most debilitated and elderly patients (Nos. 2, 3, and 6) were able to tolerate the treatments without side effects.

Tumor Response. Typically 2 weeks after EPT(B) treatment of the oral-pharyngeal neoplasm, gray-white necrotic material formed over the tumor surface (Fig 2). The necrotic material was easily debrided with suction. Pink granulation tissue began to replace tumor at around 3 weeks following treatment.

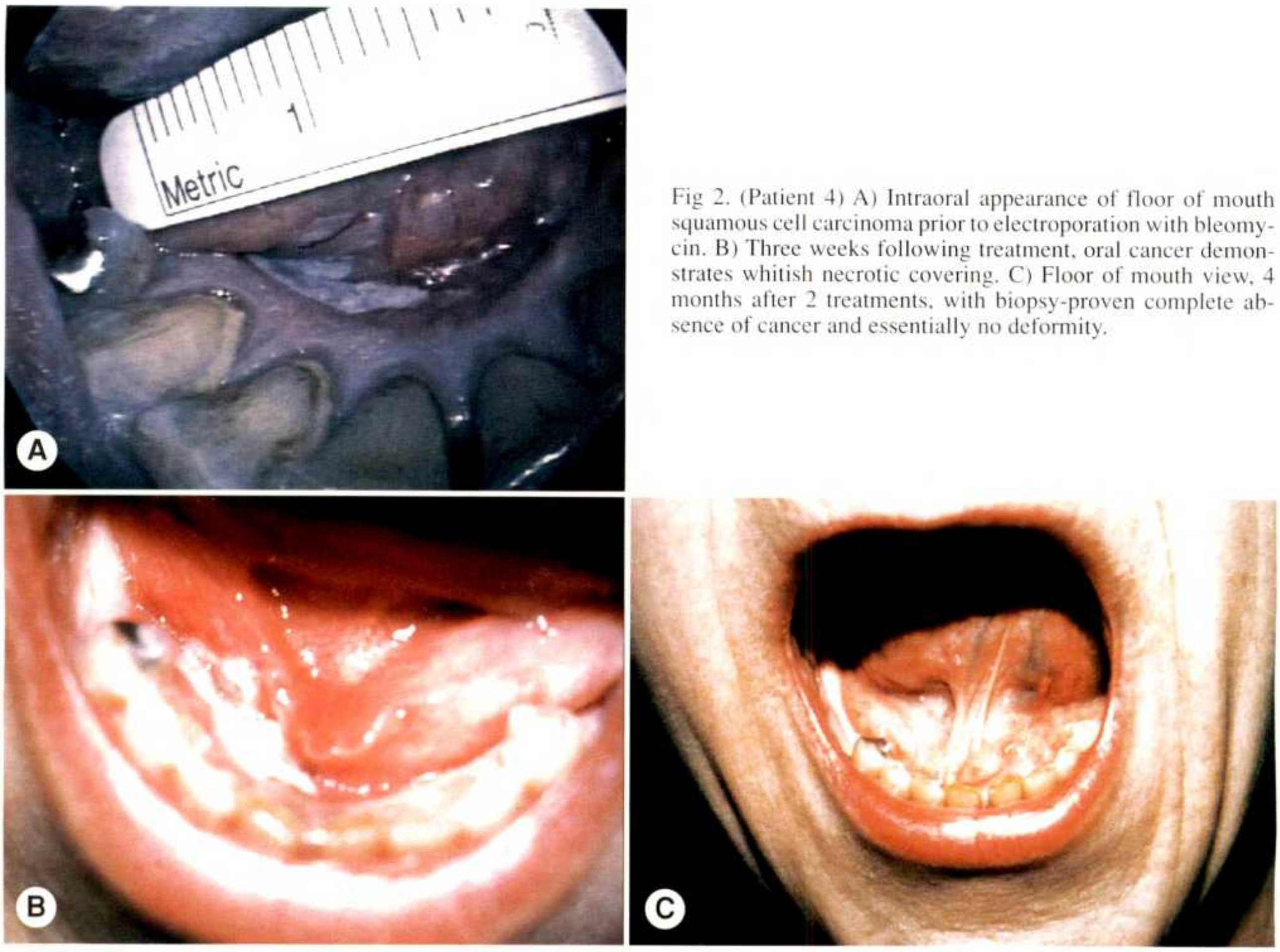

Fig 2. (Patient 4) A) Intraoral appearance of floor of mouth squamous cell carcinoma prior to electroporation with bleomycin. B) Three weeks following treatment, oral cancer demonstrates whitish necrotic covering. C) Floor of mouth view, 4 months after 2 treatments, with biopsy-proven complete absence of cancer and essentially no deformity.

On lesions that were not in contact with saliva, eg, intranasal skin surface, a black eschar formed instead of the gray-white necrotic material. Essentially only tumor cells are affected by the simultaneous application of EPT and bleomycin. Tumor cell metabolism and/or unique membrane characteristics may account for this specificity seen in this initial clinical trial (unpublished results). Patient 1, who had a nasal septal neoplasm treated and attained a complete response, was left with a nasal septal perforation at the site of tumor origin. A prior infused computed tomography scan had revealed septal tumor involvement.

Slower wound healing following EPT(B) was observed in patients who previously received radiotherapy with or without systemic chemotherapy, as compared to an untreated patient (No. 4). Bone exposure in the floor of the mouth was a source of pain in patient 3, who had previously received full course radiotherapy before EPT(B). The delayed wound healing that was observed in the previously irradiated patients appeared to be a cause for local discomfort.

Typical EPT(B) treatment reactions included soft tissue tumor necrosis and sloughing over a 4- to 8-week period. Healing was delayed in patients who had undergone previous irradiation to the head and neck areas. We were surprised to see, regardless of tumor location, minimal swelling and no bleeding following EPT(B). This was particularly noteworthy in patient 10, who had a persistent squamous cell carcinoma of the epiglottis treated with EPT(B) following irradiation and chemotherapy, and required no subsequent upper airway support.

DISCUSSION

Electroporation therapy depends on the cellular membrane phenomenon of transient micropore formation following application of a square wave electrical impulse into living tissue. Extracellular molecules (whether a chemotherapeutic agent such as bleomycin or genetic material) will then flow into the cell through relatively large cell membrane pores created by the electrical energy. Within seconds, the cell membrane pores will close, entrapping the large molecules within the cells' cytoplasm. In the present study, bleomycin concentrated within the cells' cytosol to interfere with DNA replication and produce cytotoxicity. If no cytotoxic molecules exist in the extracellular milieu, and a high-energy electrical square wave impulse is applied to tissue, no injury or cytotoxicity will occur.

Safety. The present study utilized relatively low doses per treatment (16 units or less) of intralesional bleomycin and square wave electrical energy to produce solid cancer necrosis. Objective tumor necrosis occurred at the site of treatment in 100% of the cases in which intralesional bleomycin and EPT were simultaneously applied. There was wounding and tissue necrosis where EPT(B) was applied. Tissue necrosis sloughing and delayed wound healing were more evident in those patients who had received prior irradiation to their respective tumor sites. In no case were there septic complications. Aggressive wound care and oral antibiotics provided adequate therapy for complete wound healing in all cases. Probably because of the low intralesional doses of bleomycin, no patient demonstrated any systemic toxicity, ie, renal dysfunction, pulmonary fibrosis, or blood count or platelet effect.

Neither swelling nor hemorrhage was seen in any of the EPT(B)-treated patients. All patients received steroids (10 mg Decadron intravenously) intraoperatively, as well as antibiotics. All patients were treated as outpatients. All patients continued activities, ie, eating and speaking, in a manner similar to that prior to EPT(B). No patients required a tracheostomy because of EPT(B), regardless of tumor location. One patient (No. 8) with a base of tongue cancer eventually required a gastrostomy and tracheostomy because of weight loss secondary to dysphagia and persistent cancer. This patient did not have a complete cancer response and also required additional neck surgery that subsequently produced deglutition problems.

Bleeding was not encountered in any patients following EPT(B). However, no EPT(B) treatments were done that might jeopardize the carotid or jugular vessels. No neck cancers were treated with EPT (B).

Efficacy. The results of this phase I/II trial confirm that intralesional bleomycin EPT produces head and neck cancer necrosis. The overall clinical response rate (>50% tumor reduction) in this study was 80%. Forty percent of the patients had a biopsy-proven complete response at a mean follow-up of 10 months. (Note that patient 9 demonstrated radiographic tumor resolution only after EPT(B). No biopsy was done.) This response rate is similar to the 72% local tumor necrosis and 50% complete response rate seen in 28 squamous cell cancer dermal metastases treated with a similar EPT plus bleomycin method.[14] Subsequently, Belehradek et al[20] treated 8 patients with a total of 40 dermal squamous cell cancer metastases utilizing intravenous bolus bleomycin (10 mg/m^2) followed by EPT to the tumor nodules. Twenty-three (57%) of the nodules demonstrated a complete response.[20] Dermal cancer metastases are similar to skin cancers. Similarly, Glass et al[16-18] have demonstrated a greater than 90% response to intralesional bleomycin and EPT treatment of skin cancers, including basal cell carcinoma and cutaneous melanoma. Electroporation therapy plus intralesional bleomycin appears to be an effective treatment modality for localized solid malignant neoplasms.

Disadvantages and Advantages. Electroporation therapy requires the use of an instrument (probe) that must be inserted into the tumor. Similarly, a chemotherapeutic agent is placed within the substance of the neoplasm rather than through the vascular system. Thus, tumor access for EPT and cytotoxic infiltration can be potential problems. To properly access upper aerodigestive tract cancers, as done in this study, requires rigid endoscopy competence. The EPT probe has been necessarily modified from its original straight configuration and rather large diameter (>1 cm) to different angles with various needle lengths and smaller diameters. These probe changes have allowed relatively easy access to most of the upper aerodigestive tract. In our earlier cases, a lateral rhinotomy was necessary to gain access to tumors involving the nasal cavity and anterior skull base. Since probe modification, the nasal and paranasal sinuses, nasopharynx, and endolarynx have all become readily endoscopically accessible without the need for tissue incisions.

Infiltration of bleomycin solution into the tumor requires hypodermic needle access to the neoplasm. A uniform-as-possible distribution of bleomycin throughout the tumor usually requires multiple needle passes with judicious infiltration of the cytotoxic solution. Since a finite volume of solution will be injected into the neoplastic tissues, a 25-gauge or smaller lumen needle of sufficient length attached to a 1-mL tuberculin syringe has to date provided the best infiltration technique. A method to determine a uniform distribution of bleomycin throughout the tumor would appear to be helpful for possible better neoplastic kill. In the present series, most patients required a second EPT(B), possibly related to incomplete bleomycin tumor infiltration. Also, in a few patients the tumor site was overinjected with bleomycin because of escape of some of the cytotoxic solution from the tumor's surface. These problems appear to be primarily a technical disadvantage, since there was an 80% clinical tumor response to EPT(B) and no systemic toxicity.

Another disadvantage with EPT, regardless of the presence of a chemotherapeutic agent, is the transient masticator–skeletal muscle spasm associated with the EPT electrical pulsations. The 1 head and neck cancer patient who had EPT(B) completed under local anesthesia only did experience considerable alarm and jerking that prevented precise probe application into the tumor. This patient (No. 7) had a poor clinical response to EPT(B), possibly partially related to incomplete treatment of the tumor. Of interest, no pain is associated with the EPT(B) or the muscle spasms. The intensity of muscle spasm has been significantly reduced with recent modifications of the electrical generator used for EPT. Even though skin cancer patients have been primarily treated with local anesthesia only, head and neck tumor patients, especially those requiring a transoral-endoscopic approach, appear to be best managed with general anesthesia.

Although our experience is limited, obvious advantages of EPT over conventional ablative oncologic techniques are apparent. There has been no hemorrhage or significant swelling following EPT(B). The EPT(B) can be quickly completed (<15 to 30 minutes) in an outpatient setting. There has been minimal postoperative pain.

The EPT(B) requires limited technical expertise. Any competent otolaryngologist–head and neck surgeon familiar with upper aerodigestive tract endoscopy should, with limited instruction, be able to perform EPT(B) of head and neck neoplasms.

The EPT(B) is in itself a local form of cancer treatment, similar to surgery using laser ablation, cautery, or cold steel. Unlike surgery, EPT(B) does not immediately remove the cancer, but causes in situ tumor necrosis with subsequent wound healing. The depth of penetration and subsequent tumor destruction (2 cm or more), with minimal swelling, neoplastic specificity, and no apparent systemic toxicity, offer distinct advantages over cryotherapy and phototherapy.

Like surgery, EPT(B) can be combined with other oncologic therapy. If amenable to neck therapy, ie, surgery or irradiation, a primary such as one located within the tongue could be treated with EPT(B) and the neck could be treated with surgery or irradiation. Actually, a similar scenario was seen in patient 3, in whom EPT(B) was used for a floor of mouth cancer and brachytherapy (iridium implants) was used for a fixed metastatic cervical malignancy. In patient 4, a floor of mouth cancer was treated with EPT(B) and a secondary cancer in the lung was treated with surgery.

Unlike conventional cancer surgery, but similar to systemic chemotherapy, EPT(B) produces a localized, progressive (over weeks) necrosis of treated tissues. The EPT(B) wounding of tissues with subsequent gradual tumor destruction, although a change in treatment paradigm for the cancer surgeon, may be important to long-term cancer control. In earlier work with prostaglandin inhibitors and the use of cryotherapy and/or wounding of head and neck cancers, unexpected complete tumor destruction was elicited, suggesting a possible immunologic role.[21] More recently, Mir et al[22] demonstrated an increased rate of complete responses in animals that received daily administration of interleukin-2 in conjunction with initial EPT. They hypothesized that the host's immune system was playing an integral role in cancer destruction. The combining of EPT with intralesional chemotherapy of cancer with immune modulation may offer exciting complementary advantages in malignant tumor eradication.

Electroporation therapy in combination with intralesional bleomycin offers a new method for treating head and neck cancer. Simplicity, tumor specificity, and minimal side effects are encouraging factors for the continued investigation of EPT with intralesional cytotoxic agents. Because of these results, as well as those of a larger skin cancer trial (>90% response), US, Canadian, and European multicenter phase II/III clinical trials have been initiated to study the response to EPT(B) among head and neck, liver, and pancreas cancers, as well as Kaposi's sarcoma and melanoma.

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EXTERNAL RHINOPLASTY APPROACH FOR EXTIRPATION AND IMMEDIATE RECONSTRUCTION OF CONGENITAL MIDLINE NASAL DERMOIDS

LEILA A. MANKARIOUS, MD
BOSTON, MASSACHUSETTS

RICHARD J. H. SMITH, MD
IOWA CITY, IOWA

Surgical extirpation is the treatment of choice for nasal dermoids in the pediatric population. Approaches include vertical-dorsal rhinotomy, lateral rhinotomy, transverse rhinotomy, and external rhinoplasty. While short-term results suggest that the last approach is cosmetically most acceptable, the required wide undermining of the nasal tip and dorsum may affect subsequent nasal growth. To assess long-term results, we reviewed our experience with 8 cases encountered over the past 10 years. Modifications of the technique to minimize midline dorsal depression are described.

KEY WORDS — external rhinoplasty, nasal dermoid, surgical approach.

INTRODUCTION

Midline nasal dermoid cysts are rare congenital anomalies that occur in the general population with an estimated prevalence of 1 in 20,000.[1,2] The typical presentation is that of a sinus tract, mass, or both located at any point from the nasal root to the nasal tip. Sinus tracts usually follow the supraseptal groove created by the fusion of the quadrilateral cartilage and the upper lateral cartilages, and pass under the nasal bones to the foramen cecum. An intracranial communication is found in 36% of cases.[3]

The insidious growth of nasal dermoids, the risk of meningitis, and the cosmetic deformity are indications for surgical extirpation. Although the most widely used approach is vertical dorsal rhinotomy, other approaches include lateral rhinotomy, transverse rhinotomy, and external rhinoplasty.[4] With each technique, an attempt is made to minimize wide undermining of nasal skin to obviate secondary growth restriction. Removal of the dermoid can leave a tissue defect that affects projection of the nasal dorsum. Our long-term follow-up of the external rhinoplasty technique suggests that this approach has minimal effect on subsequent nasal growth. The concomitant placement of costal cartilage recreates the nasal dorsum and enhances the postoperative result.

MATERIALS AND METHODS

Patients. Eight children ranging in age from 11 months to 14 years (average and median age, approximately 4 years) at the time of surgery underwent an external rhinoplasty approach to excise a nasal dermoid. The average time of follow-up was 5 years (Fig 1). Long-term follow-up was obtained in all patients, although in 3 cases, evaluation was made of full face photographs, including frontal and profile views, obtained by a professional photographer because these patients lived over 1,000 miles (1,600 km) from our institution.

Technique. Proposed skin incisions along the caudal edge of the lower lateral cartilages and across the columella are marked and injected with an epinephrine-containing solution. A transcolumellar incision then is made approximately 1 to 2 mm anterior to the flared free ends of the mediocrura as a "V" or a "stair step" that connects the marginal incisions (Fig 2). Continued elevation in the subdermal plane over the nasal dorsum exposes the dermoid, its tract, and the surrounding cartilaginous vault. A small elliptical incision is made to free the tract from the overlying skin. Undermining is continued along the nasal dorsum to the nasal frontal angle, preserving the periosteum over the nasal bones.

The tract most commonly follows the supraseptal

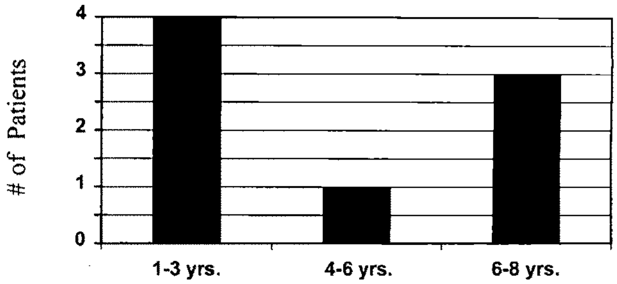

Fig 1. Graph showing time interval from surgical resection to most recent follow-up.

From the Department of Otolaryngology–Head and Neck Surgery, The University of Iowa Hospitals and Clinics, Iowa City, Iowa.

CORRESPONDENCE — Leila A. Mankarious, MD, Massachusetts Eye and Ear Infirmary, Dept of Otolaryngology, 243 Charles St, Boston, MA 02114.

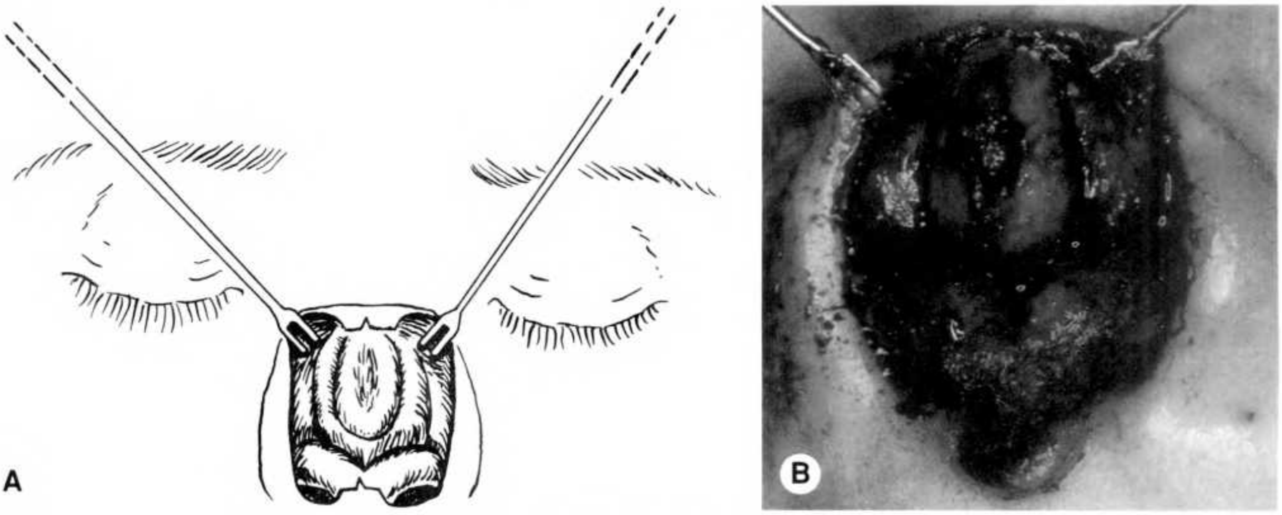

Fig 2. Initial skin incisions and soft tissue undermining. A) Schematic. B) Case 3.

groove formed by the junction of the upper lateral cartilages with the quadrilateral cartilage before coursing beneath the nasal bones to the foramen cecum. Mobilization usually is easy, and in combination with a bicoronal flap to expose the floor of the anterior cranial vault, total excision is possible.

The resulting deficit of nasal projection secondary to the splay of the upper lateral cartilages and nasal bones can be minimized by augmentation with costal cartilage. In general, the shape of the cartilage graft is that of a pyramid with a rectangular base. The base of the pyramid is placed underneath the skin envelope and the peak is placed between the splayed lateral cartilages and nasal bones. The longest dimension of the rectangular base is situated along the length of the dorsum of the nose. In our grafts, perichondrium was left on the cartilage graft along the "dorsal" surface or base of the pyramid. The skin envelope is usually enough to hold the graft in place. Incisions are closed with 6-0 fast-absorbing gut, and a pressure dressing is applied over the nose.

CASE REPORTS

Case 1. A 4-year-old girl presented with the pit of a nasal dermoid located at the radix of the nose. In January 1990, the dermoid was excised by an external rhinoplasty approach. The defect was reconstructed with costal cartilage. Shown are her preoperative

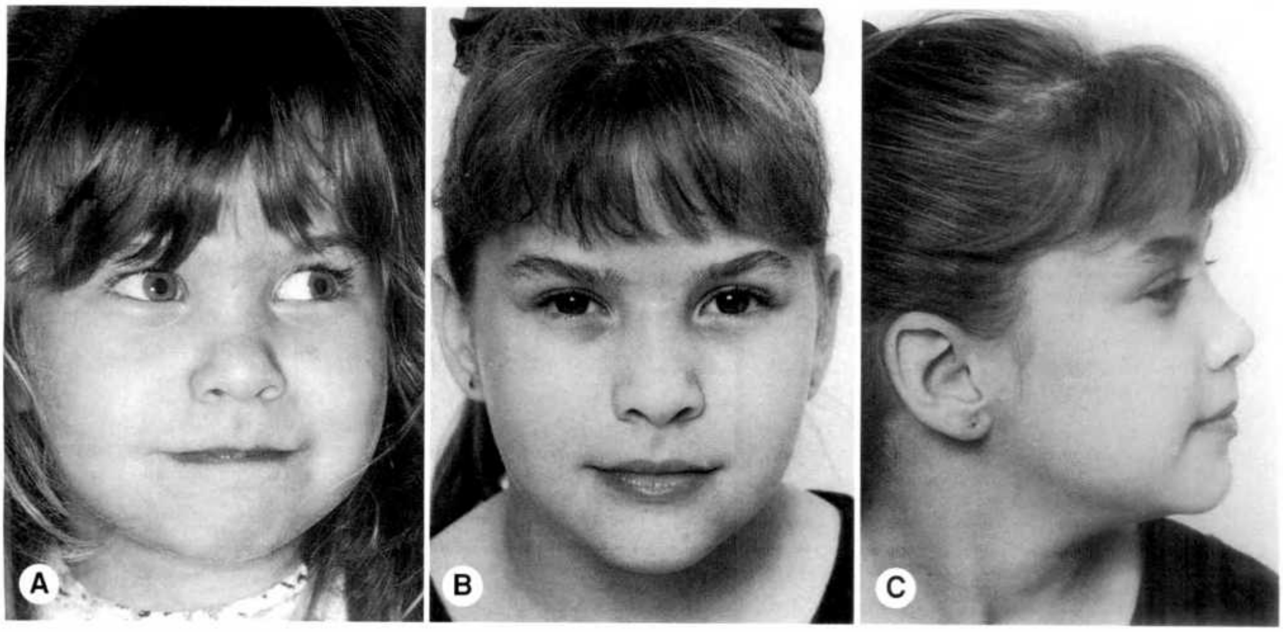

Fig 3. (Case 1) A) Preoperative photograph, age 4, showing nasal dermoid as pit in superior aspect of dorsum. B,C) Seven-year postoperative views, age 11, showing good nasal projection and minimal scarring. Scar along nasal dorsum is from excision of nasal dermoid's skin communication.

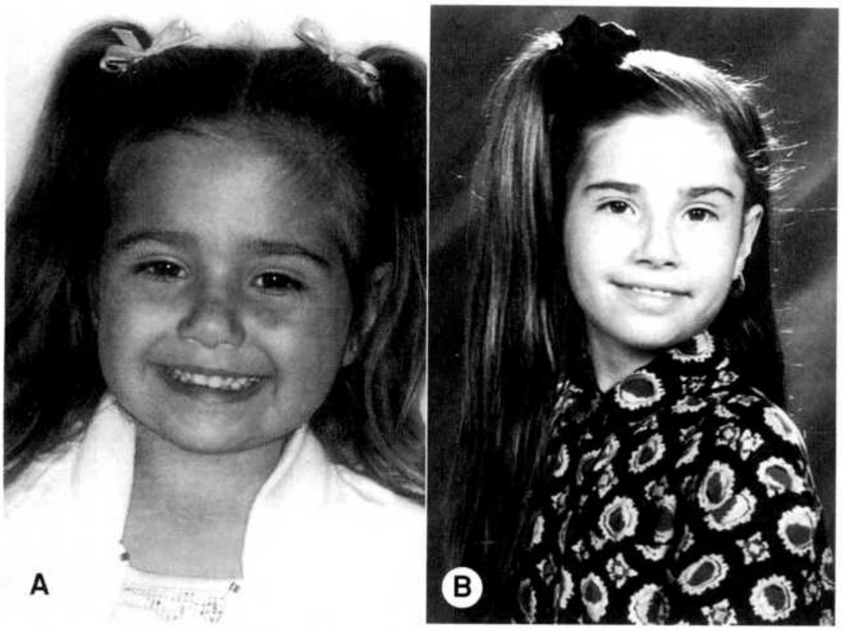

Fig 4. (Case 2) A) Preoperative photograph, age 4, showing nasal dermoid as pit along inferior aspect of nasal dorsum. B) Seven-year postoperative view, age 11, showing appropriate nasal projection and minimal noticeable scar.

and 7-year postoperative photographs (Fig 3).

Case 2. A 4-year-old girl presented with a nasal dermoid just superior to the supra-tip region with an intracranial extension. She underwent complete excision of the mass by a combined coronal–external rhinoplasty approach followed by immediate reconstruction of the nasal dorsum with a costal cartilage graft. Shown are her preoperative and 7-year postoperative photographs (Fig 4).

Case 3. A 2-year-old girl presented with a nasal dermoid cyst located along the nasal dorsum near the nasal tip. The slowly growing cyst had been present since birth and had a small intracranial communication demonstrable by computed tomography scanning. Excision of the cyst by external rhinoplasty and frontal craniotomy approaches was followed by immediate augmentation of the dorsal depression with a costal cartilage graft (Fig 5).

RESULTS

No patient developed a recurrence, and there was no instance of postoperative infection, bleeding, cerebrospinal fluid rhinorrhea, or stenosis of the nasal vestibule. In each case, the only visible scar on the nasal dorsum was that created by excision of the opening of the dermoid sinus. Typically, this scar was small compared to the overall size of the dermoid. The scar across the columella was nearly invisible after approximately 6 months.

Cartilage grafts remained appropriate augmentation for nasal projection, even through pubescent growth of the nose into its adult form. In no instance did the cartilage graft warp or change its shape to an appreciable degree.

CONCLUSIONS

The external rhinoplasty approach provides excellent exposure to the nasal dorsum and does not hamper complete excision of nasal dermoids. As cases 2 and 3 demonstrate, even dermoids with significant intracranial components can be excised readily. Sinus tracts can be followed easily and removed in their entirety.

With meticulous technique, postoperative scarring is minimized, especially over the nasal dorsum. The benefit of a subdermal approach is exemplified by case 3. Although this dermoid could have been removed easily by a dorsal rhinotomy, by using an external rhinoplasty approach, scarring was nearly avoided. In only 1 case (case 1) is there a perceptible scar over the nasal dorsum.

The increased undermining necessitated by an external rhinoplasty approach may impact on subsequent nasal growth. Extrapolation of results from studies of lateral nasal wall and septal surgery suggests that some type of growth disturbance would not be unexpected.[5,6] However, the follow-up data available to us suggest that if there is a growth disturbance, it is relatively minor. All of our patients appear to have appropriate nasal projection for age, albeit with costal cartilage grafts for augmentation of the nasal dorsum. Given the quality of these cosmetic results, the exposure afforded by this type of approach, and the lack of significant morbidity, we believe that the external rhinoplasty technique is the preferred method for the excision of congenital midline nasal dermoids.

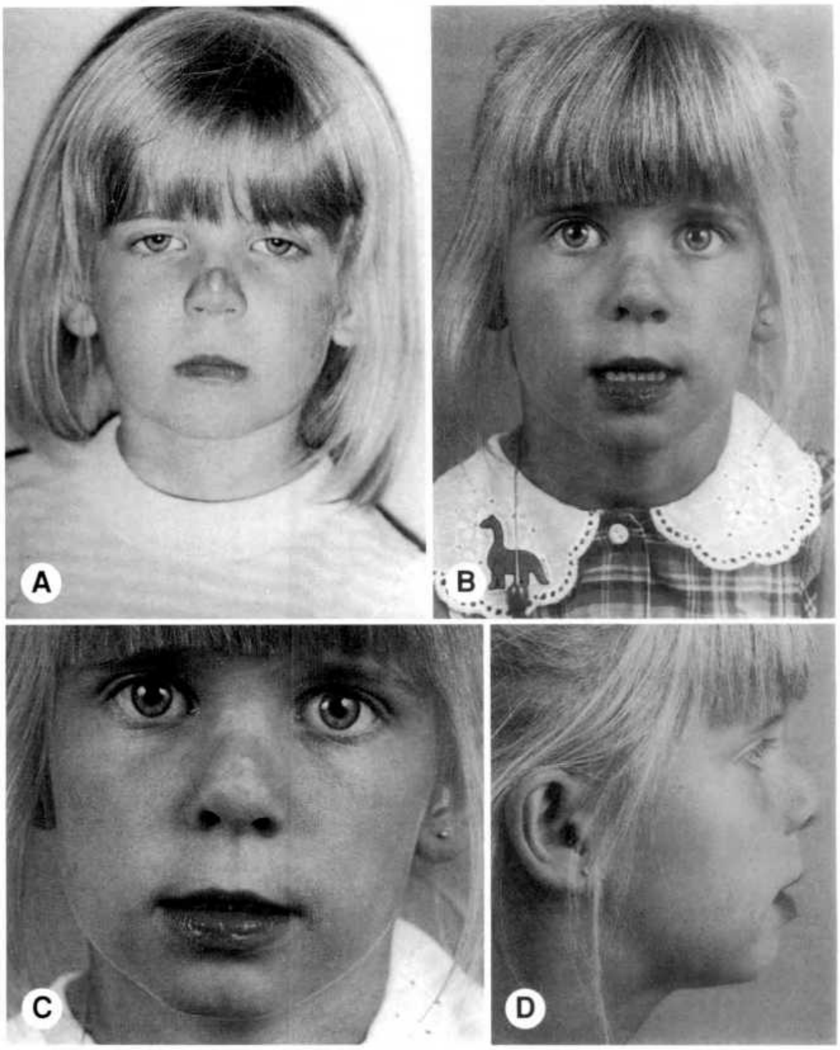

Fig 5. (Case 3) A) Preoperative view, age 2, with nasal dermoid as seen. B-D) Three-year postoperative photographs, age 5, showing appropriate nasal projection and minimal perceptible scar along nasal dorsum.

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TRACHEAL NEOPLASMS IN CHILDREN

DEVANG P. DESAI, MD
LAUREN D. HOLINGER, MD FRANK GONZALEZ-CRUSSI, MD
CHICAGO, ILLINOIS

Primary tracheal neoplasms are extremely rare lesions in the pediatric age group. This study reviews the English-language literature to better characterize these lesions in children and reports 2 additional patients. Reports of only 36 infants and children through adolescence with primary tracheal neoplasms were discovered after an exhaustive literature review of the last 30 years. The data are analyzed with regard to pathology, demographics, symptomatology, site, and percent luminal obstruction. We report 2 additional patients with photographic documentation, imaging studies, and histopathology. Of the 36 previously reported lesions, 64% were characterized as benign and 36% as malignant. Fifty-six percent of all lesions were initially misdiagnosed as asthma. The most common site was the posterior membranous wall of the cervical trachea. In 14 (39%) of the 36 patients, the lesions obstructed more than 50% of the lumen at the time of diagnosis. The timely diagnosis of tracheal masses depends upon maintaining a high index of suspicion and conducting an efficient workup, including definitive evaluation by bronchoscopy. The evaluation and the differential diagnosis of tracheal neoplasms in the pediatric population is discussed.

KEY WORDS — children, primary neoplasms, tracheal neoplasms.

INTRODUCTION

Tracheal neoplasms are rare lesions, particularly in the pediatric age group. We describe 2 cases of pediatric tracheal tumors that had been initially misdiagnosed as asthma. Both cases are presented in detail with full photographic documentation, including airway radiographs, endoscopic photography, and histopathology. Diagnosis of these and other tracheal masses depends on maintaining a high index of suspicion complemented by a selective workup.

The incidence of primary tracheal neoplasms is estimated to be less than 0.2 per 100,000 persons per year.[1] Only 8% of these tumors occur in children, 7% of which are malignant.[2] In 1949, Gilbert et al[3] reviewed the literature and reported 41 tracheal tumors in children, including 2 of their own. Since this report, there has been no comprehensive review of primary tracheal neoplasms in children.

METHOD AND MATERIALS

A 30-year (1965 to 1995) Medline search of the English-language literature was conducted. One hundred articles reported only 36 cases of primary tracheal neoplasms in children within the 30-year period. The relevant reports were reviewed to obtain data regarding pathology, symptomatology, site, and percent luminal obstruction. Review of the records at The Children's Memorial Hospital for the same period of time disclosed 2 additional lesions, which are presented in detail.

CASE REPORTS

Case 1. A 10-year-old girl presented with a history of obstructive sleep apnea and exercise-induced asthma. Her symptoms had progressed over the previous 6 months despite 1 year of medical therapy with inhaled steroids and bronchodilators. Upon examination in the emergency room, the patient had little respiratory distress, with mild biphasic stridor and wheezing. The patient had more airway obstruction when supine, but improved in the knee-chest position. The rest of the examination was unremarkable. Posteroanterior and lateral chest radiographs were normal. She was scheduled for elective bronchoscopy within 48 hours.

Following uneventful induction of anesthesia, bronchoscopy revealed a smooth, sessile, yellow lesion emanating from the posterior tracheal wall 2 cm below the vocal folds, occluding 95% of the airway (Fig 1A). A midline vertical incision was made through the fifth, sixth, and seventh tracheal arches and an airway was established by placement of an anode endotracheal tube below the lesion. Esophagoscopy findings were normal. The tracheal incision was extended superiorly through the cricoid cartilage, revealing the extent of the lesion from the posterior cricoid to the fifth tracheal arch. Frozen section biopsy yielded a diagnosis of granular cell tumor. The lesion was removed by electrocautery dis-

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From the Department of Otolaryngology–Head and Neck Surgery, University of Illinois at Chicago College of Medicine (Desai), and the Division of Pediatric Otolaryngology (Holinger) and Department of Pathology (Gonzalez-Crussi), The Children's Memorial Hospital, Chicago, Illinois.

CORRESPONDENCE — Lauren D. Holinger, MD, The Children's Memorial Hospital, Division of Pediatric Otolaryngology, 2300 Children's Plaza, Box 25, Chicago, IL 60614.

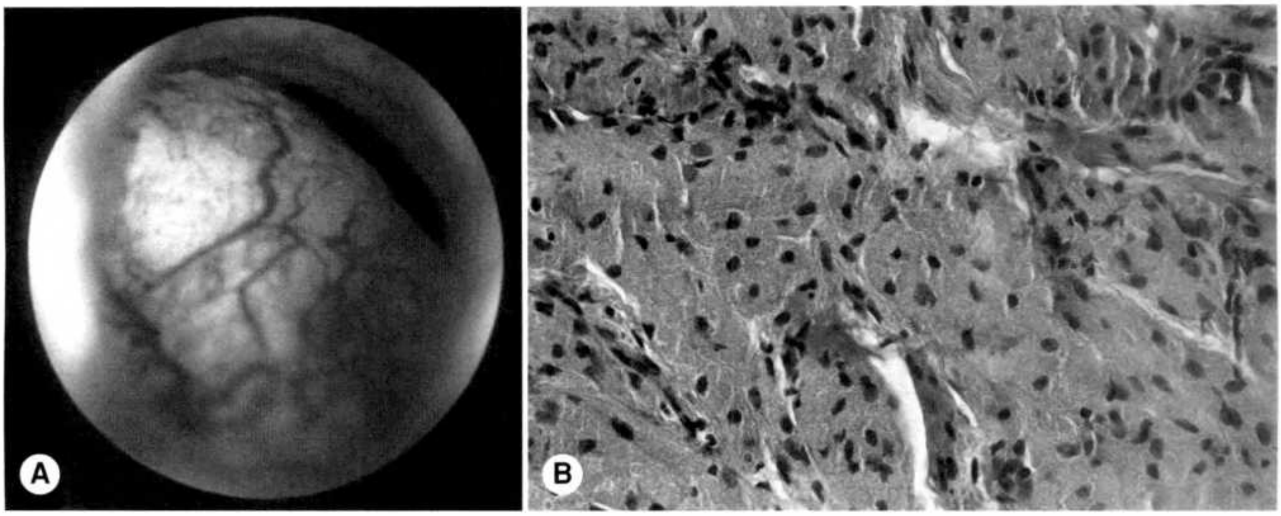

Fig 1. (Case 1) A) Endoscopic photograph of midtracheal granular cell tumor. Lesion is based on posterior tracheal wall and occludes 95% of lumen of cervical trachea. B) Typical histologic appearance of granular cell tumor, characterized by closely aggregated cells with abundant, coarsely granular, eosinophilic cytoplasm (H & E, original ×400). Nuclei are generally round and uniform, without atypical features.

section. Final pathology revealed large monomorphic cells, noted to be polygonal, with numerous small, intracytoplasmic granules. There were no malignant changes noted (Fig 1B). The patient has not had a recurrence over the following 6-year period.

Case 2. A 4-year-old girl was initially evaluated by her pediatrician for new-onset wheezing. She was started on bronchodilators and oral steroids for a presumptive diagnosis of asthma. She was returned to the emergency room four times within a 6-month period with wheezing and airway distress. She was admitted three of those times for pneumonia.

When first seen at Children's Memorial Hospital, she was in moderate respiratory distress with retractions and wheezing. She was most comfortable while lying in the right or left decubitus position rather than supine. The patient had moderate relief with racemic epinephrine, bronchodilator, and steroid therapies.

Posteroanterior and lateral chest radiographs were normal. A computed tomography (CT) scan revealed a 2 × 1 × 1-cm mid-tracheal lesion without extraluminal spread or tracheal cartilage erosion (Fig 2A).

Bronchoscopy confirmed the lesion to be 4 cm above the carina, pedunculated, and based on the posterior tracheal wall, occluding greater than 90% of the airway (Fig 2B). The lesion was submitted to biopsy; by frozen section, it was interpreted as a granular cell tumor. The lesion was excised with a ventilating resectoscope with blended cutting and coagulating current.[4] The final reading of a low-grade mucoepidermoid carcinoma was confirmed by special stains (Fig 2C,D). Repeat bronchoscopy with biopsies of the area 8 weeks later was negative for recurrence.

RESULTS

Table 1[5-37] summarizes the histopathology of primary tracheal neoplasms in the pediatric age group reported during the last 30 years. Of the 36 previously reported lesions, 23 (64%) were characterized as benign and 13 (36%) as malignant. Among the benign lesions, hemangioma and granular cell tumor were the most common. Benign fibrous lesions included 1 case of mesenchymoma and 3 cases of benign fibrous histiocytoma. Mucoepidermoid carcinoma and malignant fibrous histiocytoma were the most commonly reported malignant lesions. Malignant fibrous lesions included 1 case of fibrosarcoma and 3 cases of malignant fibrous histiocytoma.

The age at time of diagnosis ranged from the newborn period to 18 years. All 5 cases of hemangioma presented within the first few months of life. Eleven of the 13 malignant cases presented during adolescence. Fifty-seven percent of the tracheal neoplasms occurred in males, and 35% occurred in females. The sex of 2 patients with benign lesions, both hemangiomas, was not reported. Of the malignant tumors, 4 (31%) occurred in males and 9 (69%) occurred in females.

Of the published cases, 20 (56%) presented with wheezing for a period of months to 1 year and were initially misdiagnosed as asthma (Table 2[5-37]). Other common presentations include 13 (36%) with stridor, 12 (33%) with cough, and 9 (25%) with dyspnea. Three cases, all fibrous histiocytomas, presented with hemoptysis. One case each of fibrous histiocy-

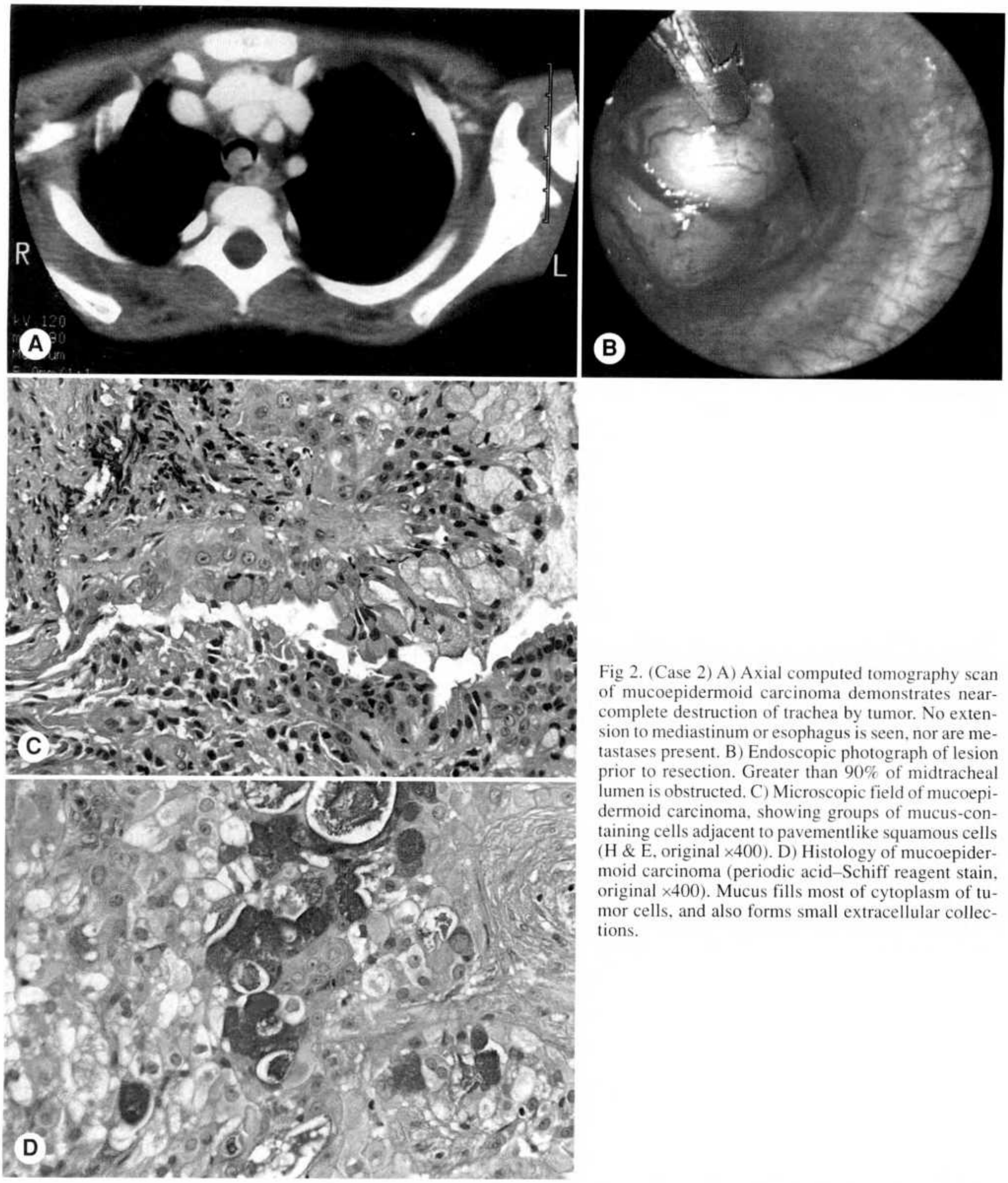

Fig 2. (Case 2) A) Axial computed tomography scan of mucoepidermoid carcinoma demonstrates near-complete destruction of trachea by tumor. No extension to mediastinum or esophagus is seen, nor are metastases present. B) Endoscopic photograph of lesion prior to resection. Greater than 90% of midtracheal lumen is obstructed. C) Microscopic field of mucoepidermoid carcinoma, showing groups of mucus-containing cells adjacent to pavementlike squamous cells (H & E, original ×400). D) Histology of mucoepidermoid carcinoma (periodic acid–Schiff reagent stain, original ×400). Mucus fills most of cytoplasm of tumor cells, and also forms small extracellular collections.

toma and granular cell tumor presented with pneumonia. For 1 case, a granular cell tumor, no data on symptoms were available.

Table 3[5-37] tabulates the location of the 36 reported neoplasms: 16 in the cervical trachea, 12 in the intrathoracic trachea, and 1 in both (hemangioma). The precise location was not recorded in 7 cases. Of the benign lesions, most (52%) were in the cervical trachea. Of the malignant lesions, the majority (54%) were in the intrathoracic trachea.

Data regarding position of the tumor on the tracheal wall were available for only 12 cases. Two tumors (17%) arose from the anterior tracheal wall, 8 (67%) from the posterior wall, and 2 (17%) from the

TABLE 1. PRIMARY TRACHEAL TUMORS IN CHILDREN

	No. of Patients	*%*
Benign		
Hemangioma	5	
Granular cell tumor	4	
Benign fibrous	4	
Papilloma	3	
Neurofibroma	3	
Pleomorphic adenoma	2	
Hemangioendothelioma	1	
Neurilemoma	1	
Total benign	23	64
Malignant		
Malignant fibrous	4	
Mucoepidermoid carcinoma	3	
Adenoid cystic carcinoma	2	
Rhabdomyosarcoma	2	
Squamous cell carcinoma	1	
Carcinoid	1	
Total malignant	13	36
Total	36	100

Information is from references 5-37.

lateral wall. For the 14 cases with available data, the tumors at presentation obstructed anywhere from 50% to 95% of the tracheal lumen.

Pulmonary function tests were obtained in 5 patients, ages 12 to 18 years. A CT scan of the chest was obtained in 7. Bronchoscopy was done for all 36 patients. Tracheal resection was the definitive treatment used for 19 patients. Endoscopic excision was the next most common treatment modality (10 patients). Tracheal fissure with wide local excision was utilized in 5 cases.

DISCUSSION

Symptomatology is related to location within the trachea and percent of obstruction caused by the tumor. Cervical tumors in juxtaposition to the vocal folds cause noisy respirations. Intrathoracic tumors adjacent to the bronchi cause wheezing and signs of atelectasis mimicking asthma.[38]

Intrathoracic tumors of the mid-trachea may go unrecognized until large enough to cause significant symptoms.[39] It has been noted that 50% to 75% tracheal obstruction is necessary before the onset of symptoms. In 14 of the 36 tumors for which data were available in our review, the lesions obstructed from 50% to 95% of the lumen at the time of presentation.

Initial nonspecific symptoms may include a persistent brassy cough and dyspnea, especially that induced by exertion.[38] The cough is initially dry, but later becomes productive due to retained secretions. A sensation of tickling in the throat may also be elicited.[38] Hemoptysis may also occur, usually with malignant tumors. Hoarseness may occur in advanced stages of malignant tumors due to involvement of the recurrent laryngeal nerves. Dysphagia is a rare symptom, but can occur secondary to extrinsic compression of the esophagus by the tracheal mass.[39]

TABLE 2. SYMPTOMS AT PRESENTATION

Symptom	*No. of Patients*	*%*
Wheezing	20	56
Stridor	13	36
Cough	12	33
Dyspnea	9	25
Hemoptysis	3	8
Pneumonia	2	6
Unrecorded	1	3

Information is from references 5-37.

The physical examination may reveal other subcutaneous granular cell lesions or neurofibromas for those cases suspected to be granular cell tumors or neurofibromas within the trachea. Also, a thorough history or examination may disclose cutaneous hemangiomas.

Signs or physical manifestations of a tracheal mass can include stridor and wheezing. This report demonstrates that 36% of the cases presented with stridor. Also, 56% of the cases presented with wheezing and were initially misdiagnosed as asthma. Other studies also emphasize that wheezing was the most common complaint in patients with tracheal neoplasms.[40] As a result, the average delay in accurate diagnosis is 10 months.[38] Our 2 cases had been misdiagnosed as asthma as well. Jackson[40] referred to "asthmatoid wheezing," an asthmalike breathing heard at the patient's mouth. Such breathing indicates obstruction of the larger air passages, as caused by tracheal luminal obstruction.[40]

The differential diagnosis of such mimics of asthma includes bronchial or tracheal compression from vascular anomalies and neoplasms arising from the mediastinum, tracheal stenosis, airway foreign bodies, reactive airway disease, and tracheobronchial

TABLE 3. LOCATION

	Benign		*Malignant*	
Location	*No. of Patients*	*%*	*No. of Patients*	*%*
Cervical	12	52	4	31
Thoracic	5	22	7	54
Both	1	4	0	0
Unknown	5	22	2	15

Information is from references 5-37.

neoplasms.[40] Jackson's[40] aphorism "all is not asthma that wheezes" suggests the possibility of a tracheal tumor when the "asthmatoid wheeze" is heard. Symptoms of progressive dyspnea with little or no response to bronchodilators and steroid therapy need to be further evaluated. In addition, diagnoses that merit further workup include recalcitrant asthma and recurrent pneumonias.

The workup may begin with radiography of the airway. Posteroanterior and lateral airway radiographs (soft tissue, high-kilovoltage, magnified radiographs) outline the tracheal air column. Radiographs of the chest may demonstrate pneumonia or atelectasis. Computed tomographic imaging of the neck and chest may help define the intraluminal extent of the lesion, extraluminal spread to the esophagus or mediastinum, tracheal cartilage erosion, and metastases.

Optional studies include barium esophagram, particularly if there are symptoms of dysphagia, in order to rule out extrinsic compression or invasion of the esophagus. An angiogram may be helpful if a vascular tracheal tumor is suspected.[41] In cooperative children, pulmonary function studies can demonstrate evidence of obstruction with reduced flows and forced volumes. In particular, the flow-volume curve is sensitive in detecting obstructive lesions of the large airways.[5]

The final diagnosis relies on bronchoscopy. Direct laryngoscopy and bronchoscopy are undertaken to further assess the extent of the lesion, to establish a safe airway, to perform a biopsy on the mass, and to rule out multiple lesions.

The literature suggests that primary pediatric tracheal neoplasms are quite rare. A review of 100 articles relevant to this literature and a Medline search from 1965 to 1995 disclosed only 36 cases. However, this figure underestimates the total number of primary tracheal tumors in children. Grillo and Mathisen[42] analyzed the treatment results of 12 other tracheal neoplasms in children; their cases are not included, because of the paucity of specific data.

A major review by Gilbert et al,[2] covering the years between 1908 and 1952, disclosed 42 reported tracheal tumors in children, of which 23 were papillomas, 9 fibromas, 6 hemangiomas, 1 an osteochondroma, and 3 sarcomas. In contrast, our review showed only 3 cases of primary tracheal papillomas, and no cases reported as fibromas. It may be that this discrepancy is attributable to reporting bias; that is, as some lesions became more widely known, interest in reporting them decreased. It is also of interest that our review yielded 64% benign tumors, whereas in the review of Gilbert et al, fully 90% of pediatric tracheal tumors were benign. Whereas reporting bias may also account, at least in part, for this disparity, changing concepts on the pathologic classification of neoplasms are probably just as important. For instance, the concept of "fibrohistiocytoma" as a tumor that is partly histiocytic, or of dual fibroblastic-histiocytic nature, had not been established during the period covered in the Gilbert et al review. This concept evolved slowly, and enjoyed widespread acceptance among pathologists in the 1970s and early 1980s; in more recent years it has been questioned, so that an authoritative recent treatise on soft tissue tumors states that "the term *fibrohistiocytic* is descriptive, and merely denotes a lesion composed of cells that resemble normal histiocytes and fibroblasts."[43(p293)]

Tumors composed predominantly of spindle cells identified as fibroblasts or myofibroblasts by current histologic, immunohistologic, and ultrastructural techniques constitute a heterogeneous group of lesions whose classification is still in a state of flux. In children, fibroblastic or myofibroblastic proliferation during reactive processes or during healing may be severe enough to simulate a neoplasm. It is thus of interest to note whether the patients with tumor-like tracheal masses were subjected to previous tracheostomy, a clinical datum that is not always recorded. Only 1 case was diagnosed as fibrosarcoma in our review. It has been proposed that fibrous tumors of the tracheobronchial tree constitute a special category, with distinctive histologies and patterns of growth.[35] This claim is yet to be substantiated; however, it is generally known that pediatric fibrous tissue tumors of relatively benign histologic appearance, in various anatomic locations, may present as masses that locally recur and invade, even though they may lack metastasizing potential.

Regardless of histology, tracheal tumors are the cause of considerable morbidity, and may determine mortality, due to their critical location. Moreover, a lack of correlation between histology and prognosis is observed in some tumors, such as carcinoids or granular cell tumors. Such neoplasms are sometimes assigned in the literature to a category of "intermediate" or "indeterminate" prognosis. Therefore, it may be difficult, in some cases, to decide on the benign or malignant nature of a tracheal tumor. In the present review, we opted to follow the reported histologic diagnosis, and thus, granular cell tumors were placed in the benign group, and the single carcinoid tumor in the malignant group.

Another interesting difference between the previous major review of the literature by Gilbert et al[2] and the present one is the emergence of carcinomas. The reported tracheal carcinomas were mucoepidermoid, squamous, and adenoid cystic; all are very uncommon, even in the adult. A squamous cell carcinoma occurred in a 10-month-old boy without any apparent risk factors. Adenoid cystic carcinoma and carcinoid tumors occur more commonly in the bronchi, although they are generally quite rare. Similarly, the occurrence of mucoepidermoid carcinoma is well documented in the bronchi of children,[44] and we have added here 1 observation of a tracheal mucoepidermoid tumor in a 4-year-old girl. This is a low-grade malignancy, apt to recur locally but unlikely to metastasize, save in exceptional instances.

Gilbert et al[3] reported that the sites of occurrence within the trachea, in increasing order of frequency, are upper third, lower third, and middle third. In contrast, our review found the cervical trachea to be the most common site of occurrence. In addition, this study demonstrates that malignant lesions occurred more commonly in the intrathoracic rather than the cervical trachea. This finding is corroborated by the observation that malignant lesions in adults occur in the lower trachea, especially the supracarinal location, and certainly much more commonly in the bronchial tree. Although the trachea appears to be immune from risk factors that predispose to malignancy, the bronchial tree is much more susceptible, possibly due to chronic exposure to carcinogens.[42] Our analysis demonstrates that the posterior wall is the most common tracheal position involved. Both of our cases originated from the posterior tracheal wall.

The mainstay of therapy for tracheal neoplasms is surgical excision. Since the review includes both benign and malignant lesions, no discussion of therapy could be all-inclusive. Tracheal fissure for local excision was utilized in 5 cases. Endoscopic excision was performed in 10 cases. Tracheal resection was utilized in 19 cases. One resection was for a sarcoma with no response to combination chemotherapy and radiotherapy. Another was utilized for a malignant fibrous histiocytoma that failed endoscopic excision. Follow-up is dictated by clinical history, CT scans, and serial bronchoscopy with biopsies as necessary.

CONCLUSION

A review of the English-language literature for the last 30 years discloses 36 tracheal neoplasms in children. We report 2 additional cases. Although respiratory symptoms in children secondary to asthma or foreign bodies are common, there is a possibility of a tumor in a child masquerading as asthma or recurrent pneumonia. The timely diagnosis of tracheal masses depends upon maintaining a high index of suspicion and conducting an efficient workup, including definitive evaluation by bronchoscopy. The importance of endoscopic assessment is emphasized in order to define the extent of the mass, to rule out multiple lesions, to establish and maintain an airway, and to perform a biopsy of the mass.

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PHARYNGEAL FIBROVASCULAR POLYP IN A CHILD

MERRITT J. SESHUL, MD
CARLOS A. GALLIANI, MD
BRIAN J. WIATRAK, MD
GREGORY T. ODREZIN, MD
BIRMINGHAM, ALABAMA

Fibrovascular polyp of the upper aerodigestive tract is an uncommon tumor that may present in pediatric patients with symptoms ranging from dysphagia to asphyxiation and death. We present a unique case of a pediatric patient with an asymptomatic fibrovascular polyp noted as an incidental finding on a cervical ultrasound evaluation. This lesion extended from the posterior tonsillar pillar and prolapsed freely into the nasopharynx and esophagus. The literature relevant to this case is reviewed, and the etiology, pathophysiology, and management principles are discussed.

KEY WORDS — fibrovascular polyp, pharynx.

INTRODUCTION

The combination of esophagoscopy and recent advances in telescopic video capabilities has allowed a more precise description and categorization of pharyngeal and esophageal lesions. Fibrovascular polyp is a benign lesion, usually of the pharynx or esophagus, which has tremendous potential to cause airway obstruction due to its mobility. While unusual in adults, this lesion is even less common in children. We present a case of a pharyngeal fibrovascular polyp in a child that arose from the posterior tonsillar pillar and prolapsed into the esophagus. This lesion was asymptomatic at presentation and was an incidental finding.

CASE REPORT

A 5-year-old girl with a history significant for reactive airway disease, allergic rhinitis, and atopic dermatitis presented for evaluation of a small left neck mass. On physical examination, a compressible, mobile, subcutaneous cervical mass (level II) was noted. Sonography revealed this mass to be cystic. However, as an incidental finding, a second, deeper cervical mass that was bilobed, mobile, and echogenic was noted behind the left lobe of the thyroid gland, in the region of the esophagus. A computed tomography (CT) scan revealed the mass to have fat density (Fig 1A). A subsequent barium swallow study revealed an intrinsic, mobile, lobulated mass in the cervical esophagus arising from or invading the lumen (Fig 1B). Careful review of the history divulged odynophagia and frequent coughing. On the basis of the clinical history and radiographic findings, esophagoscopy was scheduled. At that time, a firm, fleshy, mucosal-covered polyp was noted to extend from a stalk arising from the posteroinferior aspect of the left tonsillar pillar (Fig 1C). This pendulous mass prolapsed freely both down into the esophagus and high into the nasopharynx. Inspection to the gastroesophageal junction showed no other mucosal lesions or masses. At this point, elective bilateral tonsillectomy was performed with electrocautery. The stalk of the freely mobile mass was then transected at the level of the posterior tonsillar pillar with electrocautery. The patient tolerated the procedure well. Postoperatively, the parents recalled a past episode of eructation of a fleshy mass that the child quickly swallowed. The patient was discharged the same day and has not returned for excision of the superficial cystic neck mass.

PATHOLOGY

The specimen from the left tonsillar pillar consisted of a 3.1-g, 3.9 × 1.1 × 1.0-cm, pedunculated, pink-tan, smooth to finely granular, rubbery to doughy polyp with an elongated, bilobed head. The cut surface exhibited a uniform white-yellow core (Fig 2A).

Histologically, a myofibrovascular stalk connected the base to a core of mature adipose tissue, the entire structure covered in part by a metaplastic stratified squamous epithelium. Focal epithelial hyperplasia was noted (Fig 2B). Focally, a ciliated goblet cell containing pseudostratified epithelium was identified, with a discontinuous band of lymphocytes and plasma cells in a well-vascularized lamina propria. Occasional clusters of seromucous glands were ran-

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From the Department of Surgery, University of Alabama at Birmingham (Seshul, Wiatrak), and the Departments of Pediatric Otolaryngology–Head and Neck Surgery (Seshul, Wiatrak), Pathology (Galliani), and Pediatric Imaging (Odrezin), The Children's Hospital of Alabama, Birmingham, Alabama.

CORRESPONDENCE — Brian J. Wiatrak, MD, Dept of Pediatric Otolaryngology–Head and Neck Surgery, The Children's Hospital of Alabama, 1600 Seventh Ave S, ACC 320, Birmingham, AL 35233.

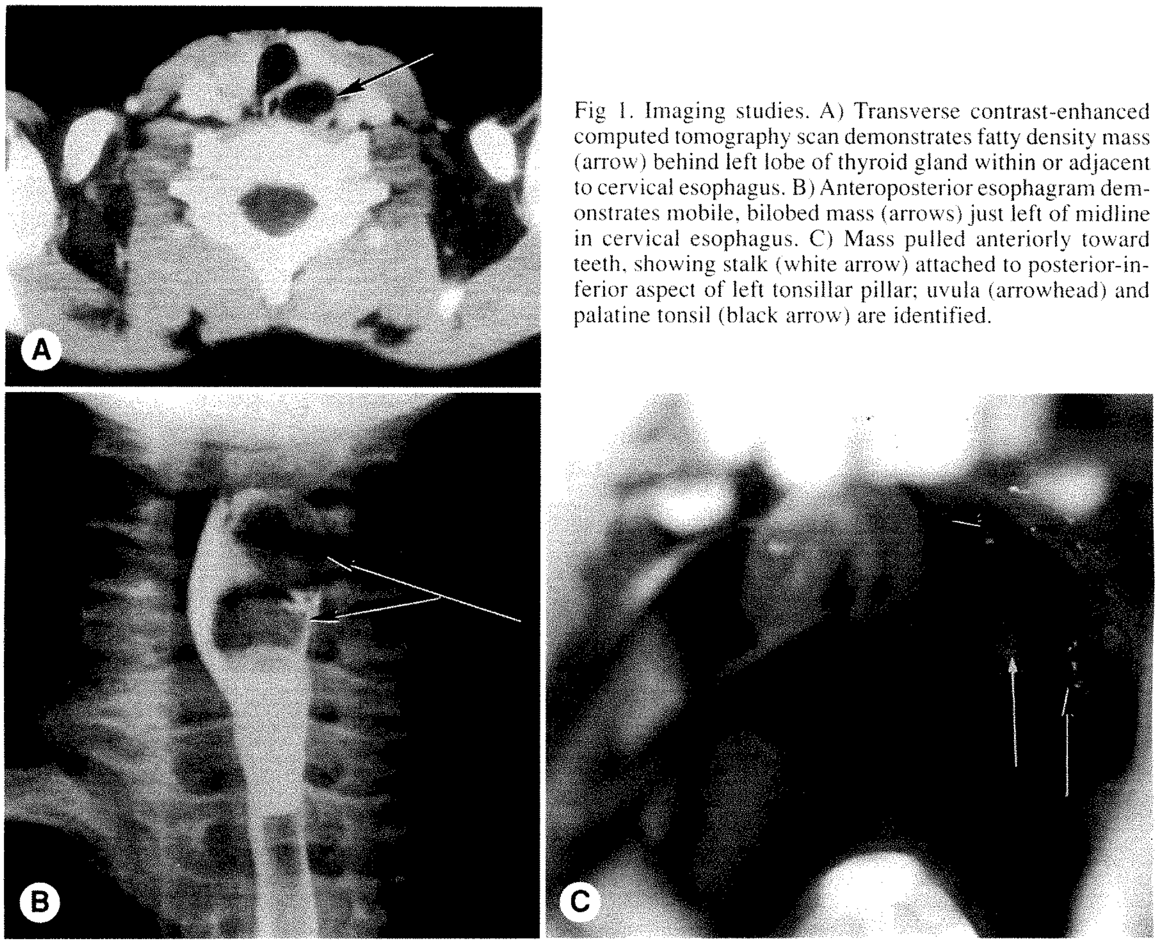

Fig 1. Imaging studies. A) Transverse contrast-enhanced computed tomography scan demonstrates fatty density mass (arrow) behind left lobe of thyroid gland within or adjacent to cervical esophagus. B) Anteroposterior esophagram demonstrates mobile, bilobed mass (arrows) just left of midline in cervical esophagus. C) Mass pulled anteriorly toward teeth, showing stalk (white arrow) attached to posterior-inferior aspect of left tonsillar pillar; uvula (arrowhead) and palatine tonsil (black arrow) are identified.

domly distributed in the submucosa.

DISCUSSION

Most esophageal masses in adults are malignant neoplasms, while only 20% are benign.[1] These lesions may be extraluminal, intramural, or intraluminal. Intramural leiomyoma is the most common benign esophageal neoplasm. Benign intraluminal lesions are most commonly fibrovascular polyps or squamous papilloma, the former being more common.[2] Fibrovascular polyps are more common in adults than in children, tending to arise in the cervical esophagus in adults and from the oropharynx in children.[3,4] Theisen[5] and others have reported rare

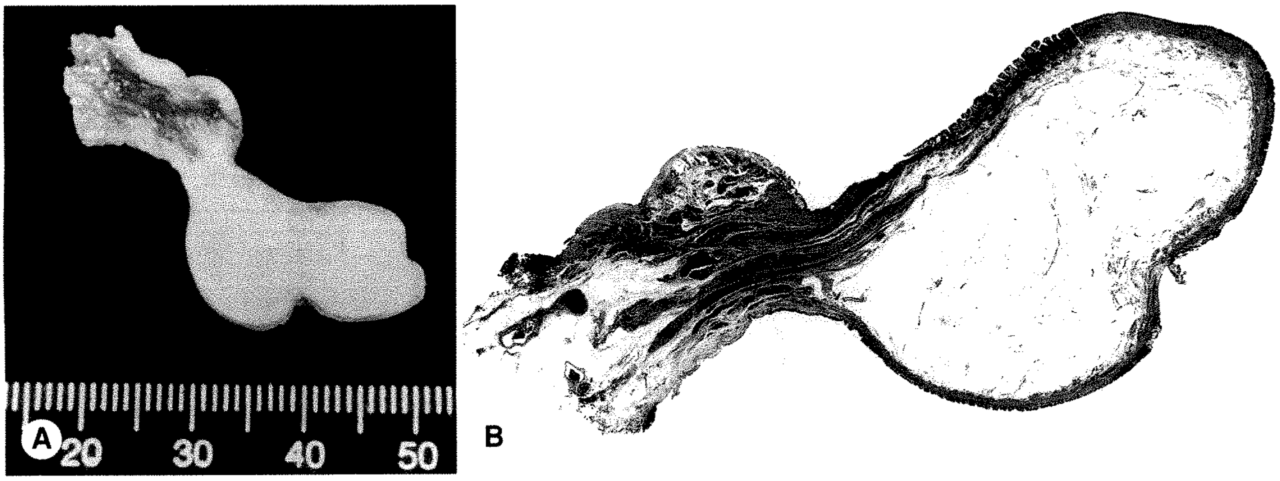

Fig 2. Pathology. A) Pedunculated, bilobed polyp with uniform white-yellow core on cut surface. B) Histologic section demonstrates core of mature adipose tissue covered by stratified squamous mucosa with papillae and ciliated, goblet cell–bearing epithelium.

pediatric cases. Rare reported pediatric cases arose from the tonsillar pillar.[6,7] A variety of terms have been used to describe a fibrovascular polyp, including benign esophageal polyp, fibrolipoma, pedunculated lipoma, myxoma, and combinations of these terms. Fibrovascular polyps were first described in 1893 by Biaggi, and the multiple cases since reported have indicated a broad spectrum of clinical presentation.[8,9] The symptomatology of fibrovascular polyps is diverse, ranging from asymptomatic to obstructive asphyxia.[10] Other manifestations may include dysphagia, odynophagia, nausea, substernal discomfort, foreign body sensation, eructation of mass, cough, dyspnea, voice changes, sonorous snoring, and aspiration with laryngeal obstruction. Careful workup and a high index of suspicion are required, as these lesions are lined with normal mucosa and may be difficult to appreciate with esophagram, CT scan, or endoscopy. Surgical excision is paramount, due to the risk of aspiration and airway obstruction. Indeed, there are several reported cases of death as the initial presentation, due to prolapse of this lesion into the respiratory tract.[10] Upon diagnosis of this lesion, airway management is top-priority; elective tracheotomy preceded excision in some reported cases.[11] Additionally, these masses may bleed or undergo malignant degeneration.[10,12] Both of these complications may be exacerbated by gastroesophageal reflux. The surgical approach is dictated by lesion location, with thoracotomy rarely being required. Careful follow-up is necessary, as recurrence has occurred up to 15 years after excision.[13]

The variety of presentations of this lesion explains the many descriptive terms in the literature. In addition, it begs the question of the origin of this lesion. Though fibrovascular polyps have been recognized for a century, their exact cause and pathogenesis remain elusive. They can arise from any of multiple sites in the aerodigestive tract, including the pharynx,[14] tonsil,[5] postcricoid region and esophagus,[15] larynx,[16] bronchus,[17] parapharyngeal space,[18] and oral cavity (cheek).[4] Thus, there have been attempts to explain pathogenesis on the basis of area of origin. Owens et al[11] surmise the pathophysiology of those polyps arising from the upper third of the esophagus to parallel the development of a Zenker's diverticulum. Laimer's triangle is the area of the posterior and most superior portion of the cervical esophagus. This region is inherently weak due to splaying of the posterior longitudinal muscle fibers to insert anteriorly behind the cricoid cartilage.[19] Tonic cricopharyngeal contraction and esophageal peristaltic waves are theorized to cause nodular submucosal thickenings or redundant folds. These folds would then be further molded into enlarging polyps by peristalsis.[20] Another theory is that local injury and inflammation may damage elastic fibers in the submucous layer of the pharyngeal or esophageal wall, thereby initiating a similar process.[21] Theisen[5] in 1903 postulated that ectopic embryonal fatty cells were the cause. More recently, the fibroblast has been touted as a multipotential cell that may facultatively differentiate into a fat cell.[13] Of note, fibroblasts and preadipose cells both derive from undifferentiated mesenchymal cells. Although morphologically indistinguishable, these cells are distinct entities.[11] One question is whether these lesions represent ectopic fibroblasts or preadipose cells, an abnormality in their growth regulation, or a combination of these factors. Another theory of the genesis of these lesions is that they are first branchial cleft anomalies representing choristomatous heterotopic "ears."[22] Support for this theory lies in the number of cases arising from the nasopharynx and the presence of elastic cartilage in many of these lesions. This histologic finding is in contradistinction to the histologic examination of teratomas, which very rarely reveals elastic cartilage. Unfortunately, the reported case does not seem to fit neatly into one of these theories of causation.

CONCLUSION

Fibrovascular polyps are benign pharyngeal and esophageal lesions primarily reported in adults. Although benign, they are considered "malignant" by some, due to their ability to prolapse freely from their site of origin and cause respiratory compromise and even complete obstruction. No definitive pathogenesis has been determined to date, and one must wonder if separate causes for pediatric and adult lesions exist. Careful review of history, physical examination, and radiographic studies, as well as a high index of suspicion, are required for diagnosis. Establishing a stable airway is important. Surgical excision is the treatment of choice. Prolonged follow-up is needed due to the possibility of recurrence.

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CLINICAL AND PATHOLOGIC CHARACTERIZATION OF MUCOSA-ASSOCIATED LYMPHOID TISSUE LYMPHOMA OF THE HEAD AND NECK

NEIL BHATTACHARYYA, MD
ROBERT A. FRANKENTHALER, MD
HILARY I. GOMOLIN, MD
MARSHALL E. KADIN, MD
ARTHUR M. LAURETANO, MD
BOSTON, MASSACHUSETTS

Mucosa-associated lymphoid tissue (MALT) has recently been recognized as a possible site of origin for low-grade lymphomas of the B-cell type. Though relatively rare, these MALT lymphomas may arise within several sites in the head and neck, and often present diagnostic and therapeutic challenges. We review 4 cases of primary MALT lymphoma of the head and neck, treated with surgical excision (3 cases), irradiation (2 cases), and chemotherapy (1 case), to further characterize this new subtype of head and neck malignancy. The mean time from onset of symptoms to histologic diagnosis was 15 months. Fine needle aspiration identified an atypical lymphoid infiltrate in only 1 of 3 patients. Immunohistochemical analysis was essential in establishing the diagnosis of MALT lymphoma in all 4 of the cases, and demonstrated characteristic negative staining for CD3, CD5, and CD43, positive staining for CD20, and monotypic staining for either κ or λ light chain immunoglobulin markers. All patients achieved complete remission after primary therapy, and all remain free of disease with follow-up ranging from 6 to 54 months (mean 33 months). The diagnosis of MALT lymphoma should be considered in cases of atypical lymphoid infiltrates in the head and neck, and increased awareness coupled with detailed immunohistochemical analysis is essential to securing an accurate diagnosis. Clinical remission of MALT lymphoma may be achieved with several modalities, but further study will be required to determine the long-term response to treatment.

KEY WORDS — head and neck neoplasms, immunohistochemistry, lymphoma, mucosa-associated lymphoid tissue.

INTRODUCTION

Lymphomas constitute a small overall percentage of head and neck malignancies, but up to 70% of lymphomas may have head and neck manifestations.[1] Approximately 10% of lymphomas involve extranodal sites in the head and neck; these extranodal lymphomas may be of the Hodgkin's or non-Hodgkin's type.[2] Within the spectrum of non-Hodgkin's extranodal lymphomas, the mucosa-associated lymphoid tissue (MALT) lymphoma occupies a distinctive position because of its relative rarity, difficult diagnosis, and indolent clinical course.

The term MALT refers to aggregates of extranodal lymphoid tissue that normally reside within the submucosa of the epithelial-lined tracts of the body. Though MALT is significant in quantity, tumors of the MALT system have only recently been described as a separate type of lymphoma. The identification and classification of MALT lymphoma has been fostered by recent advances in immunoperoxidase staining and molecular techniques that allow for the subclassification of cell types and the detection of monoclonality within lymphoid infiltrates. This pathologic distinction is important in the differentiation of MALT lymphoma from other benign and malignant processes.

We describe here 4 cases of MALT lymphoma that have been diagnosed and treated at our institution in order to better delineate its clinical presentation, difficulties with its pathologic diagnosis, and its response to treatment. The literature on MALT lymphoma in the head and neck is also reviewed.

CASE REPORTS

Case 1. A 45-year-old woman initially presented with swelling at the right angle of the mandible 2 months after ipsilateral mandibular dental extractions. Over the next 1.5 years, the swelling gradually increased, and she began having pain along the inferior border of the mandible. Additionally, intermittent twitching of the right midface began. A fine needle aspiration biopsy of the fullness at the mandibular angle was consistent with reactive lymphoid tissue, and she was managed conservatively.

The facial twitching, swelling below the mandibular angle, and parotid tenderness persisted for an additional year, and the patient presented to our insti-

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From the Joint Center for Otolaryngology (Bhattacharyya, Frankenthaler, Lauretano) and the Departments of Hematology and Oncology (Gomolin) and Pathology (Kadin), Beth Israel–Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts.

CORRESPONDENCE — Neil Bhattacharyya, MD, Joint Center for Otolaryngology, 333 Longwood Ave (3rd Floor), Boston, MA 02115.

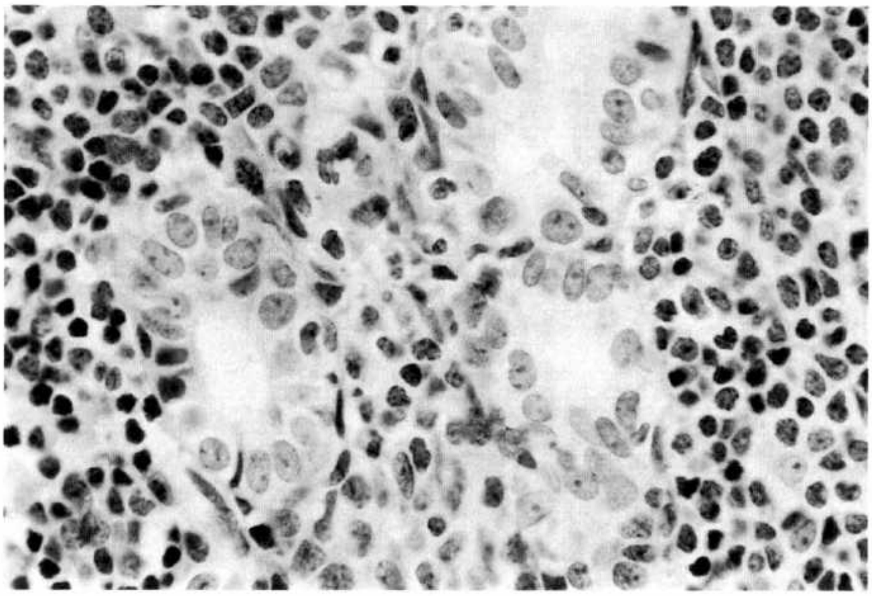

Fig 1. (Case 1) Mucosa-associated lymphoid tissue (MALT) lymphoma of parotid gland (H & E, original ×800). There are parotid ducts compressed and infiltrated by atypical lymphocytes creating characteristic lymphoepithelial lesions.

tution. The head and neck examination findings were normal with the exception of the right side of the neck. A palpable mass was noted over the lower border of the parotid, and a 1-cm right jugulodigastric node was detected. No other cervical or axillary adenopathy was appreciated. The facial nerve was intact. The patient's medical history was unremarkable, with no history of Sjögren's syndrome, familial malignancies, childhood irradiation, fever, or night sweats. Because of the persistence of the symptoms and the associated adenopathy, she underwent excisional biopsy of the node and parotid biopsy through a parotidectomy approach. Frozen section was nondiagnostic. The final pathology confirmed an atypical extranodal lymphoid infiltrate within the parotid gland (Fig 1); the lymph node was normal. Subsequent immunoperoxidase stains of the parotid tissue revealed expression of B-cell antigens with the CD20 marker and monotypic κ light chain staining of numerous small lymphocytes and plasma cells (Fig 2). Additional gene rearrangement analysis revealed clonal changes of immunoglobulin light and heavy chains consistent with MALT-type lymphoma. Computed tomography (CT) of the torso and bone marrow biopsy staging were both negative for disseminated disease, and her disease was therefore classified as stage IE. Treatment consisted of external beam irradiation to the parotid bed to a total of 3,660 cGy. The facial twitching and tenderness have resolved completely. The patient remains disease-free now, 6 months after tissue diagnosis.

Case 2. A 35-year-old man with a history of Sjögren's syndrome presented with a 2-year history of a slowly enlarging left parotid mass. A magnetic resonance imaging (MRI) scan was obtained that identi-

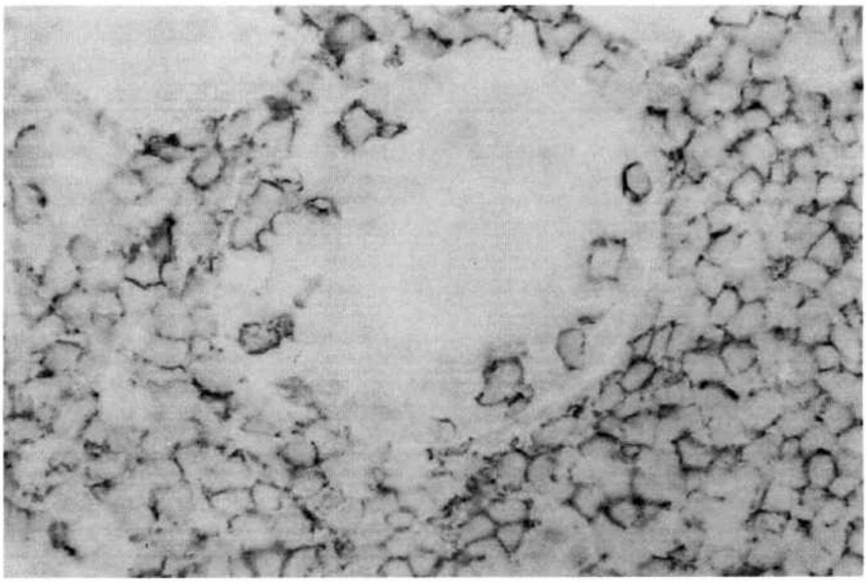

Fig 2. (Case 1) Immunoperoxidase analysis of MALT lymphoma of parotid gland (original ×800). Staining for immunoperoxidase L26 (CD20+) marker shows positive staining within lymphoma cells around parotid duct.

fied a mass in the superficial lobe of the left parotid gland, and the patient was referred for otolaryngological evaluation. Physical examination revealed a 1.5-cm left parotid mass overlying the angle of the mandible, with an intact facial nerve and a normal right parotid gland. The remainder of the head and neck examination was negative. A fine needle aspirate revealed bland-appearing lymphocytes thought to be arising from an intraparotid lymph node. Within 3 weeks, the parotid mass increased in size, and the patient underwent a standard superficial parotidectomy. Intraoperatively, a diffuse enlargement of the parotid tail was noted. The pathology of the lesion revealed a low-grade lymphoma with sheets of centrocyte-like cells, germinal centers, and follicular colonization within parotid tissue, consistent with MALT lymphoma. Supplemental immunoperoxidase studies confirmed the absence of CD3, CD5, and CD43, the presence of the CD20 marker, and λ light chain in a clonal pattern, helping confirm the diagnosis of MALT lymphoma.

The patient was evaluated with CT imaging of the neck, chest, and abdomen and bone marrow biopsy, the findings of which were normal, and the diagnosis was MALT lymphoma stage IE. The patient was then referred for radiotherapy to the parotid gland for local control of the MALT lymphoma. He received a total of 3,600 cGy to the parotid bed over the course of 3.5 weeks. He tolerated radiotherapy well, with only slight exacerbation of the dry eye syndrome. Repeat CT imaging of the neck, chest, and abdomen and repeat bone marrow biopsy have not demonstrated disseminated involvement. A gallium scan also showed no focal abnormality. The patient remains free of disease now, 54 months after treatment.

Case 3. A 56-year-old woman noted the subtle onset of swelling and tenderness in the right nasolabial fold, which was initially treated with antibiotics for a presumed maxillary sinusitis. After the swelling failed to resolve, she was referred to the otolaryngology department for evaluation. On physical examination, she was found to have a mass in the right gingivolabial sulcus, and the remainder of the head and neck examination was negative. A biopsy of the right maxillary sinus was performed via a Caldwell-Luc approach. Intraoperative findings were significant for a submucosal mass involving the anterior wall of the maxillary sinus with no bone erosion. The pathology disclosed an atypical lymphoid infiltrate with predominantly round lymphocytes and plasmacytoid cells. Immunoperoxidase studies demonstrated positive staining for B-cell antigen CD20, as well as monoclonal staining for κ light chain immunoglobulin. These immunohistochemical and histologic findings were consistent with a low-grade lymphoma of the MALT type.

As part of the standard staging procedure, the patient underwent CT scanning of the neck, thorax, abdomen, and pelvis. Though no other lymphadenopathy was identified, asymmetry of the gastric folds was noted. Therefore, the patient underwent esophagogastroduodenoscopy and biopsy of thickened gastric folds near the fundus. Again, pathology with immunoperoxidase staining revealed a MALT lymphoma histopathologically identical to that of the maxillary sinus. Her disease was therefore staged as a stage IIIA MALT-type lymphoma. As treatment, she underwent 6 cycles of cyclophosphamide and prednisone chemotherapy over the course of 4 months. Remission of the tumor in both sites was noted. On scheduled restaging 6 months after therapy, repeat gastric biopsy confirmed resolution of the thickening, but persistence of the tumor by biopsy. Because of the persistent gastric tumor, the patient underwent external beam radiotherapy to the stomach to a total dose of 3,500 cGy. (Radiotherapy was not delivered to the maxillary sinus because of the complete remission at that site.) She tolerated this well. She underwent repeat gastroscopy 1 year after radiotherapy and no tumor was found. She remains free of disease at both sites currently, 2 years after initiation of therapy.

Case 4. A 40-year-old woman presented to her internist with a 12-year history of fullness in the right parotid region, and recent onset of parotid swelling, which was felt to be due to a parotid duct stone. This spontaneously resolved until 1 year later, when the swelling returned over the right parotid, and she was referred to our institution. A fine needle biopsy was done of a discrete right parotid fullness, and suggested an atypical lymphoid infiltrate. She also noted a 15-year history of Sjögren's syndrome. For diagnostic purposes, she underwent a right superficial parotidectomy, and all abnormal tissue was removed. The final pathologic findings were consistent with a diffuse low-grade B-cell lymphoma. Additional immunohistochemical studies showed the parotid gland to be infiltrated by a monoclonal κ B-cell population, confirming a MALT-type lymphoma. A bone marrow aspirate, CT staging, and gallium scan were all negative, conferring a diagnosis of MALT lymphoma, stage IE. The patient chose not to undergo radiotherapy to the region because of the already present sicca syndrome. She has been followed with serial examination and CT imaging, with no evidence of disease at 48 months postparotidectomy.

DISCUSSION

It is only recently that MALT lymphoma has been

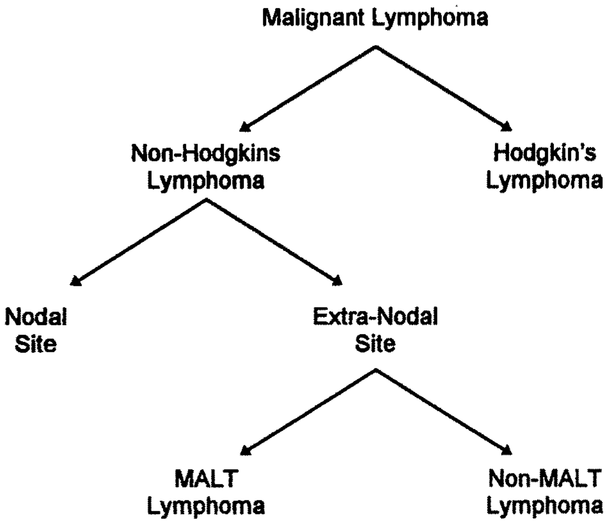

Fig 3. Classification of lymphomas by type and site of origin.

recognized and characterized as a distinct clinical entity. Recent advances in immunohistochemistry have identified MALT lymphoma as a subtype of marginal zone lymphoma, which is included in the broader group of low-grade B-cell lymphomas.[3] Clinically, MALT lymphomas arise in an extranodal site, and a useful classification scheme for these tumors is illustrated in Fig 3. Throughout the body, approximately 40% of lymphomas occur in extranodal sites, with the gastrointestinal tract being the most common site of origin.[4] The incidence of head and neck involvement by MALT lymphoma is unknown because of the relatively recent recognition of this as a subtype of low-grade lymphoma. Previously, many of these lymphoid infiltrations of mucosal tissues were likely reported as pseudolymphomas.[5,6] As noted by Horny et al,[5] the term pseudolymphoma has been used much less frequently in the literature since the emergence of the MALT lymphoma as a distinct clinical and pathologic entity.

Because there is limited information available regarding the incidence and epidemiology of MALT lymphoma in the head and neck, much of this information is extrapolated from MALT lymphoma in other sites.[7] Among all sites, MALT lymphoma is more common in females than males by a ratio of 1.4 to 1, with a mean age at presentation of 64 years.[3] The parotid gland, larynx, thyroid gland, Waldeyer's ring, and conjunctiva appear to be sites of predilection in the head and neck.[5,7,8-10] These lesions often present within the head and neck in an indolent fashion, as demonstrated by these 4 cases. A mass or submucosal thickening present for years may be the only clinical sign. The lesion is often diffuse, further complicating the diagnosis. Of interest, many of these MALT tumors occur in mucosa that is naturally devoid of MALT.[5] This suggests a possible role for the acquisition of MALT by normal tissue in response to sustained inflammation as a first stage, which then progresses to lymphomatous involvement. This may occur in sites with chronic inflammation, such as gastric mucosa infected with *Helicobacter pylori*, or presumably as in case 1, in focal chronic parotitis from dental extractions. Another well-known association of MALT lymphoma with chronic inflammation is seen in Sjögren's syndrome with the development of parotid lymphomas.[11]

The diagnosis of MALT lymphoma can be difficult because of its indolent growth and benign clinical presentation. Imaging studies are not particularly helpful other than for planning surgical biopsy; they are, however, important in staging of the lymphoma after diagnosis. Fine needle aspiration, as in cases 1 and 2, may be difficult to definitively interpret, and should not be relied on to either establish or exclude this diagnosis. Surgical biopsy of a portion of the involved extranodal tissue is the procedure of choice, but this must be coupled with careful pathologic evaluation supplemented by immunoperoxidase analysis. The biopsy material must be appropriately handled to allow for proper processing for immunoperoxidase markers. Unfixed frozen tissue may be retained by the pathologist for immunoglobulin gene rearrangement studies if needed to confirm the diagnosis. However, caution must be exercised in the interpretation of the immunoglobulin gene rearrangement results, since clonal bands are sometimes detected in cases of benign lymphoid hyperplasia as well.[12] Clonal bands representing true lymphoma can be distinguished from those occurring in benign hyperplasia by their reproducibility in deeper sections or separate tissue samples.

The recent identification of MALT lymphoma as a subtype of low-grade non-Hodgkin's lymphoma has given rise to several difficulties with the histopathology of these lesions. This arises, in part, from the multiple classification schemes for malignant lymphoma. The MALT lymphoma is considered a type of B-cell marginal-zone lymphoma, as noted by Fisher et al.[13] The marginal zone is a B-cell territory that lies immediately beyond the mantle of small B-lymphocytes that naturally surrounds the germinal center of the lymphoid follicle. A study by Dierlamm et al[14] provides additional morphologic, immunologic, and cytogenetic evidence for the common site and cell of origin of these lymphomas. Fortunately, the MALT lymphoma manifests several immunohistochemical markers that aid in its differentiation from other low-grade lymphomas. Use of these markers is essential, since differentiation

ANN ARBOR STAGING SYSTEM FOR MALIGNANT LYMPHOMA

Stage I	Involvement of single lymph node region (I) or of single extralymphatic organ or site (IE)
Stage II	Involvement of 2 or more lymph node regions on same side of diaphragm (II) or localized involvement of extralymphatic organ or site and of 1 or more lymph node regions on same side of diaphragm (IIE)
Stage III	Involvement of lymph node regions on both sides of diaphragm (III); may be accompanied by localized involvement of extralymphatic organ or site (IIIE), involvement of spleen (IIIS), or both (IIISE)
Stage IV	Diffuse or disseminated involvement of 1 or more extralymphatic sites or tissues with or without lymph node enlargement
Symptoms	"A" indicates absence of systemic symptoms; "B," unexplained fever, night sweats, or weight loss of more than 10% of body weight

Information from Jacobs et al.[2]

among the low-grade lymphomas with standard light microscopic methods is extremely difficult.[3,5]

Histopathologically, the MALT cell lymphoma characteristically exhibits a distinctive lymphoepithelial lesion. This contains medium centrocyte-type cells arranged in a marginal pattern around lymphoid follicles within the mucosa or the parenchyma of the surrounding tissues, and neoplastic lymphocytes "colonizing" reactive follicles, which can, themselves, closely mimic reactive lymphoid tissue. The resemblance to a reactive lymphoid infiltrate makes the distinction between benign and malignant lesions difficult, and the immunologic marker analysis is often required. The demonstration of negative staining for CD3, CD5, and CD43, positive staining for CD20, and monotypic staining for either the κ or λ light chain immunoglobulin with these techniques secures the diagnosis of MALT lymphoma. In some cases, light chain restriction cannot be demonstrated, and the demonstration of clonality by polymerase chain reaction amplification of immunoglobulin gene(s) is required to establish the diagnosis. Additional histopathologic and immunopathologic characteristics of MALT lymphoma are described in more detail elsewhere.[3,5]

Once the diagnosis of MALT lymphoma is made, efforts should be directed toward staging the extent of disease. This includes CT scanning of the neck, chest, abdomen, and pelvis to detect other potentially involved sites. Gallium 67 scanning, used in case 2, has also demonstrated some value in staging and follow-up.[15] Though bone marrow involvement in MALT lymphoma is not common, it should be considered because of an up to 25% chance of marrow involvement for extranodal head and neck lymphomas.[2] Most MALT lymphomas of the head and neck are Ann Arbor stage IE, involvement of a single site, with the "E" denoting an extranodal site of origin. The staging system is outlined in the Table.[2] There is, however, the possibility of multiple sites of involvement, as seen in case 3, with up to 30% of cases being initially staged with disseminated disease.[13]

The natural history of MALT lymphoma has been traditionally regarded as less aggressive than that of other low-grade non-Hodgkin's lymphomas. Many cases of localized MALT lymphoma can be followed expectantly because of their extremely slow growth. Several studies have confirmed that the survival for MALT lymphomas is better than that of other lymphomas of the B-cell derivation,[3,9,16] although survival for disseminated disease approaches that of other non-Hodgkin's lymphomas.[13] Up to 30% of these MALT lymphomas may undergo transformation into a more aggressive large cell lymphoma.[4] The better prognosis and alternative treatments for the MALT subtype of low-grade lymphoma make accurate identification and differentiation of these tumors essential.

The natural tendency for MALT lymphoma to remain localized for long periods of time makes it suitable for local forms of treatment such as radiotherapy.[2,7,17] This is our current therapy of choice for these lesions, especially for stage IE disease. For disease at multiple sites and for disseminated disease, chemotherapy utilizing alkylating agents with or without radiotherapy is employed.[13] Chemotherapy alone may also be successful, as evidenced by case 3. Of interest, there are reports of complete remission after treatment by local excision with clear margins, as seen in case 4, and this further illustrates the limited tendency for dissemination of this tumor.[16]

Survival data for MALT lymphomas of the head and neck are limited. One published report indicates that survival approximates 90% at 5 years for stage IE MALT lymphomas of the thyroid gland. This is similar to survival for other low-grade non-Hodgkin's lymphomas in the head and neck.[2] To the best of our knowledge, there are no survival data for MALT lymphoma specific for the head and neck, but it is believed that the data from other sites can be extrapolated to the head and neck.[16] This would predict survival rates of greater than 70% at 6 years.[3] Our case data are too preliminary to indicate whether radiotherapy for localized disease can be expected to provide durable clinical remission. In addition, chemotherapy may produce clinical remission even in the face of higher-stage disease. Though all 4 of our patients remain disease-free with a mean follow-up of 33 months, further follow-up will be required to determine the long-term response to treatment.

CONCLUSIONS

1. The diagnosis of MALT lymphoma should be suspected in extranodal lymphoid lesions of the head and neck. Surgically obtained tissue should be appropriately handled to allow for immunohistochemical and possible gene rearrangement analyses for MALT lymphoma.

2. Immunohistochemical techniques should be employed to determine the diagnosis of MALT lymphoma because of the differences in natural history, prognosis, and therapy between MALT lymphoma and the other lymphomas. This is especially true for cases thought to represent "pseudolymphoma."

3. MALT lymphoma may respond to radiotherapy when localized, or chemotherapy when more advanced, resulting in acceptable long-term remissions when properly diagnosed and treated. Surgical excision of well-circumscribed disease may be an alternative.

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IMAGING CASE STUDY OF THE MONTH

COMPUTED TOMOGRAPHIC FINDINGS IN TWO CASES OF CELLULITIS OF THE INFRATEMPORAL FOSSA WITH ABSCESS FORMATION

KAZUNORI NISHIZAKI, MD

TERUHIRO OGAWA, MD
HIROFUMI AKAGI, MD
KAYOKO SATO, MD
YU MASUDA, MD

OKAYAMA, JAPAN

INTRODUCTION

Cellulitis of the infratemporal fossa is difficult to diagnose by conventional physical examination because of the anatomic location of this structure. To evaluate any abscess formation in this region, the use of computed tomography (CT) has been suggested.[1,2] However, CT findings of cellulitis of the infratemporal fossa have not been well described to date. Thus, we present descriptions of two cases of cellulitis of the infratemporal fossa, along with their characteristic CT findings, to clarify the diagnostic utility of this imaging method in this disorder.

CASE REPORTS

Case 1. A 62-year-old man complained of a persistent toothache in the left upper molars despite palliative treatment by a dentist. This patient then consulted a pain clinic and received a nerve block. However, this treatment proved ineffective and the pain gradually worsened. Trismus also developed. A CT scan was then performed, and the patient was referred to our clinic. Routine otolaryngological examination did not demonstrate any abnormalities other than a limited ability to open the mouth completely. The CT images revealed the disappearance of the fat plane in the retromaxillary region and a lack of the distinct contour of the muscles of mastication (Fig 1A). Because the patient's white blood cell (WBC) count was 10,100/mL and the C-reactive protein (CRP) level was 25.2 mg/dL, antibiotic therapy was begun. Three days later, the left palatoglossal arch appeared swollen. To relieve the swelling, an incision was made at the most swollen site, and the

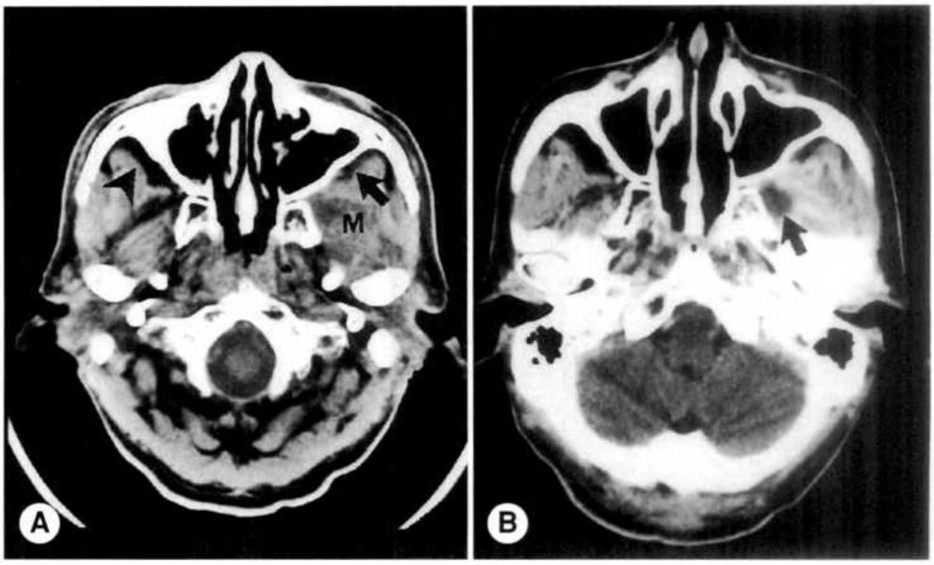

Fig 1. (Case 1) A) Axial computed tomography scan showing disappearance of fat plane in retromaxillary (arrow) and parapharyngeal regions and loss of clear demarcation of muscles of mastication (M) as compared with opposite side. Arrowhead — normal parapharyngeal fat on contralateral side. B) Enhanced axial computed tomography scan showing abscess formation (arrow) in medial portion of infratemporal fossa.

From the Department of Otolaryngology, Okayama University Medical School, Okayama, Japan.

CORRESPONDENCE — Kazunori Nishizaki, MD, Dept of Otolaryngology, Okayama University Medical School, 2-5-1 Shikata-cho, Okayama, 700, Japan.

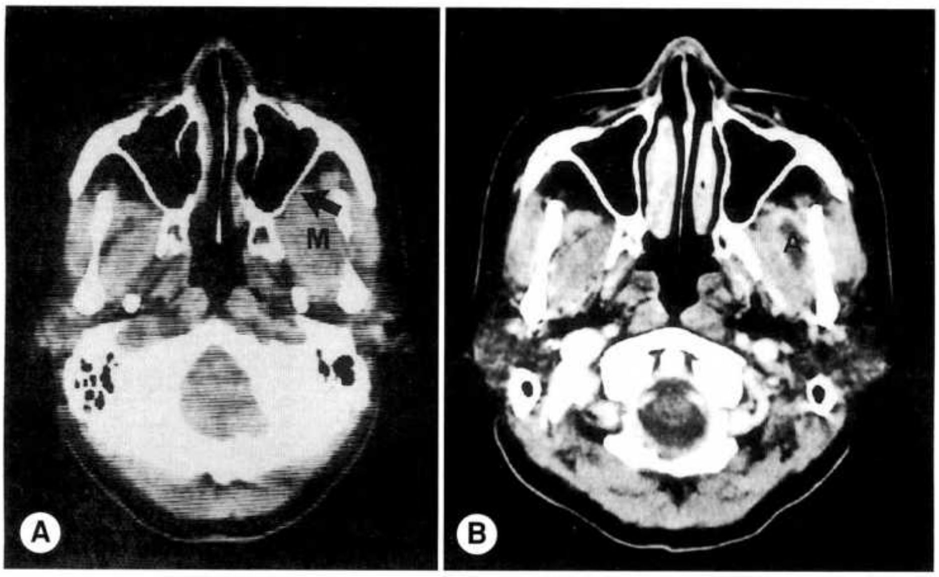

Fig 2. (Case 2) A) Axial computed tomography scan showing disappearance of fat plane in retromaxillary region (arrow) and diffuse swelling of muscles of mastication (M) as compared with opposite side. B) Enhanced axial computed tomography scan showing abscess formation (A) in lateral portion of infratemporal fossa.

fluid contents were drained. An enhanced CT scan taken 9 days after the initial CT scan demonstrated abscess formation in the infratemporal fossa (Fig 1B). Drainage and antibiotic therapy proved effective, and the patient recovered completely.

Case 2. A 57-year-old woman exhibited a toothache in the left lower molars along with gingival swelling. Although she consulted a dentist, who incised and drained the swelling, and received antibiotics, the pain persisted and trismus developed. She was referred to our clinic. Routine otolaryngological examination demonstrated mild swelling in the gingiva around the lower left second molar and in the left palatoglossal arch. A CT scan demonstrated the disappearance of the fat plane in the retromaxillary region and a lack of clarity of the muscles of mastication (Fig 2A). Since the WBC count was 7,400/mL and the CRP level was 4.3 mg/dL, antibiotic therapy was initiated. Enhanced CT images taken 3 days after the initial CT demonstrated an abscess in the infratemporal fossa (Fig 2B). The antibiotic therapy proved effective and the patient recovered completely.

DISCUSSION

The infratemporal fossa or the nasopharyngeal masticator space is a suprahyoid space that is bounded by the superficial layer of the deep cervical fascia.[3] The masticator space is divided into the superficial suprazygomatic region and the deep nasopharyngeal region. The masticator space is adjacent to the buccal space anteriorly, the parotid space posteriorly, the parapharyngeal space medially, the temporal space superiorly, and the submandibular and sublingual spaces inferiorly. Given this anatomic location, once inflammation occurs in one of the other suprahyoid spaces and breaches the fascial boundaries of the infratemporal fossa, the latter fossa is at risk for infection.[1]

Pericoronitis[2,4] sometimes spreads into the infratemporal fossa and leads to abscess formation. However, at the stage of cellulitis in which only facial pain is present, routine otolaryngological examinations usually do not reveal any abnormalities. Subsequent trismus may also make cellulitis more difficult to diagnose. Because of these diagnostic difficulties, principally due to the anatomic location of the infratemporal fossa, the pain is considered to be of unknown origin, and antibiotics are not administered. A delay in administering effective treatment may cause the infection to spread superiorly to the base of the skull and inferiorly deep into the neck — a potentially serious infection.

Computed tomography and magnetic resonance imaging make it possible to visualize the spaces within the face and neck.[5-9] In deep neck infections, it is mandatory to distinguish cellulitis from an abscess in deciding whether to treat with surgery or to use antibiotics. Although enhanced CT can delineate abscesses more clearly, plain CT is used initially as a screening test for most cases in which a deep neck infection is suspected. The findings characteristic of cellulitis on both plain CT and enhanced CT include the presence of homogeneous swelling of the soft tissue with obliteration of the adjacent fat plane.[6,10] The diagnosis of inflammation of the infratemporal fossa usually focuses on abscess formation,[1,2] not cellulitis. Before the formation of such an abscess, infiltration or obliteration of the retromaxillary and/or parapharyngeal fat and loss of clear delineation of the masticatory muscles were observed on plain CT in both of our patients. These findings resemble those observed in patients with other deep neck infections[6] or peritonsillitis.[10] In cellulitis of the infratemporal fossa, inflammatory processes, including the infiltration of inflammatory cells, stromal edema, and congestion, seem to involve the fatty tissue located within masticator muscles and increase its CT value. Once an abscess has formed, drainage from the infratemporal fossa becomes technically difficult. Therefore, when inflammation is suspected to be present deep in the neck, CT analysis should be performed, and we should pay attention not only to abscess formation but also to the disappearance of the fat plane in the retromaxillary and parapharyngeal spaces and an unclear masticatory apparatus. When such findings are present, antibiotic therapy should be initiated as soon as possible. As in the two cases described, the outlook is then favorable.

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CLINICOPATHOLOGICAL CONSULTATION

ALVEOLAR SOFT PART SARCOMA OF THE HEAD AND NECK REGION

BRIAN C. HUNTER, MD
ANN ARBOR, MICHIGAN

KENNETH O. DEVANEY, MD
ANN ARBOR, MICHIGAN

ALFIO FERLITO, MD
UDINE, ITALY

ALESSANDRA RINALDO, MD
PADUA, ITALY

Alveolar soft part sarcoma is a soft tissue malignancy most often found in the extremities of young adults; when these tumors arise in the head and neck area, they usually appear in the orbit or the tongue. Their initial behavior is relatively indolent, but over time a sizable number of these tumors recur locally and metastasize; as such, they are best regarded as fully malignant neoplasms. The derivation of these tumors remains uncertain: while some have suggested that these are tumors of muscle origin and others have postulated a neuroendocrine origin, the evidence accumulated to date is conflicting, and so these neoplasms continue to be regarded as tumors of uncertain origin. Surgical excision is the mainstay of therapy.

INTRODUCTION

Alveolar soft part sarcoma (ASPS) is a rarely encountered type of soft tissue sarcoma, comprising less than 1% of all sarcomas from all body sites in most series; as is the case with many other neoplasms, its name derives from its light microscopic appearance.[1] Most often, ASPS is a tumor of young adults, in whom the most frequent sites of involvement are the lower and upper extremities. When ASPS presents in the head and neck region, the patients involved are often younger than those affected by extremity ASPS. This report will give a typical case presentation and will survey current knowledge about this tumor. It will place particular emphasis on its presentation in the head and neck region and will serve as an update of the most recent work done to clarify the vexing question of its histogenesis.

CASE REPORT

The Figure shows the histologic features of the lesion on the tongue of a 3-year-old girl who presented in October 1992 to the University of Michigan Medical Center. Her chief complaint consisted of a mass effect with difficulty swallowing. Her chest radiographs were negative for metastases at the time of presentation, and the diagnosis of ASPS was made on biopsy material on December 11, 1992. She underwent wide local excision of this lesion in January 1993 with negative margins by frozen section. Her postoperative course was uneventful, and the patient was discharged on the third postoperative day. She was closely followed from January 1993 until January 1997 (last follow-up appointment) and has shown no evidence of metastatic or recurrent disease on physical examination or by computed tomography scans.

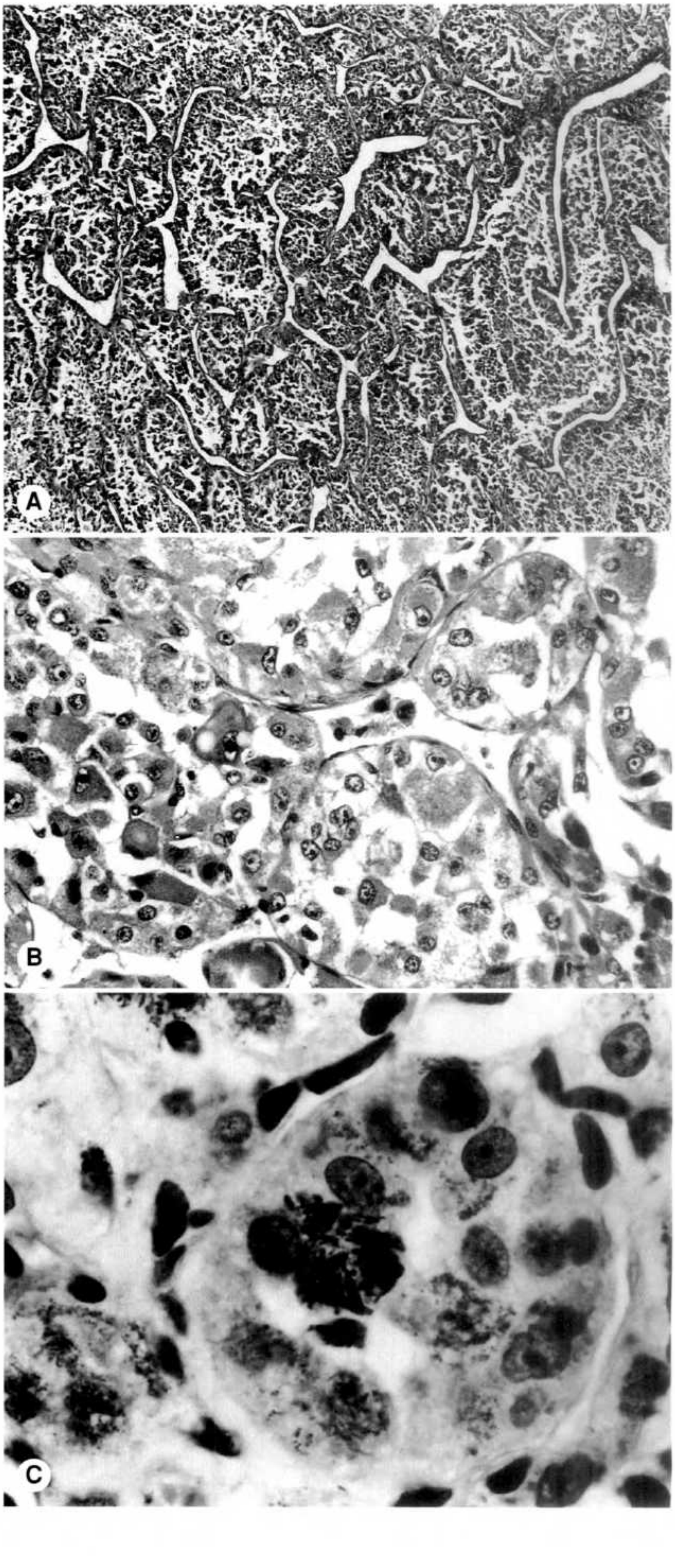
HISTOGENESIS

Prior to the 1952 report of Christopherson et al,[2] the neoplasm now designated as ASPS was known by a host of different names, including synovioma, angioreninoma, rhabdomyoma, hemangioma, liposarcoma, rhabdomyosarcoma, granular myoblastoma (malignant and benign), and malignant tumor of nonchromaffin paraganglia.[2-4] This multiplicity of names derives from the microscopic observation that these tumors consist of groups of epithelioid tumor cells set in a richly vascular matrix. Originally, the term ASPS was conceived of as a temporary designation, subject to discard after a fuller explanation of its histogenesis was developed; to date, such a fuller understanding of the nature of this tumor has not been forthcoming, and so use of the "provisional" designation persists to this day.

Unlike most other tumor types, ASPS seems to be a rather distinctive lesion in that it does not appear to have a benign counterpart. While squamous papillomas and squamous carcinomas may be taken to be the two ends of a spectrum of squamous proliferations (ranging from benign to malignant), there has not yet been identified a benign alveolar soft part tumor.

From the Department of Pathology, University of Michigan, Ann Arbor, Michigan (Hunter, Devaney), and the Department of Otolaryngology, University of Padua, Padua, Italy (Ferlito, Rinaldo).

CORRESPONDENCE — Alfio Ferlito, MD, Dept of Otolaryngology–Head and Neck Surgery, University of Udine, Policlinico Città di Udine, Viale Venezia 410, 33100 Udine, Italy.



Alveolar soft part sarcoma. A) Low-power view of typical alveolar soft part sarcoma of orbit, showing alveolar grouping of polygonal tumor cells separated by thin fibrovascular septa (H & E, original ×100). B) This alveolar soft part sarcoma of orbit shows relatively monomorphous population of polygonal cells with granular cytoplasm; mitotic figures are not apparent (H & E, original ×320). C) With use of special histochemical stain, intracytoplasmic crystals may be identified in approximately three quarters of alveolar soft part sarcomas (periodic acid–Schiff with diastase, original ×400).

While many theories have been developed regarding the histogenesis of ASPS, the identity of the tissue type from which ASPS derives remains a mystery. Of all the theories generated, the myogenic theory of origin is the one most popular at the present time, although it is not the sole line of thought in this area; our understanding has been hampered in particular by reports of conflicting data. For example, one group described ultrastructural evidence of myelinated axon formation within ASPS and so postulated a neural origin[5]; another group found no such evidence of axonal differentiation.[6,7] Moreover, with regard to the possibility that these tumors may be of neural or neuroendocrine-paraganglionic derivation, it has been demonstrated by some investigators that ASPS lacks neuron-specific enolase (NSE), P2 and P0 proteins (peripheral nerve myelin protein), S-100 protein, tropomyosin, and renin. It has been shown by formaldehyde-induced fluorescence that catecholamines are absent, and by biochemical methods, it has been further confirmed that these tumors do not produce renin.[8,9] In contrast, other observers have described NSE positivity in ASPS — although this finding is limited by the fact that this particular immunohistochemical marker is not as specific as its name would imply.[10]

With regard to the possible skeletal muscle origin of ASPS, workers agree that the tumor cells do not appear to contain myoglobin, a component of normal skeletal muscle.[8,11] However, myoglobin is a marker of well-differentiated muscle, and if ASPS is indeed derived from a myoid precursor, it may not achieve this level of differentiation. Another cytoplasmic component of many muscle tumors, desmin, has been reported to be both present[8,10,12] and absent[13] in different authors' hands by immunohistochemistry. Similarly, myogenic regulatory proteins, including MyoD1 and myogenin, have been claimed to be both present[14] and absent[15] in ASPS.

Thus, half a century after its definitive description, the histogenesis of ASPS remains a true pathologic mystery. Perhaps the recent discovery of cytogenetic abnormalities at the 17q25 position will provide new avenues for research into the mystery of this tumor's derivation.[16,17]

CLINICAL AND RADIOGRAPHIC FEATURES

Alveolar soft part sarcomas are rare neoplasms in general (0.4% to 1.0% of all soft tissue sarcomas) and occur predominantly in adolescents and young adults (ages 15 to 35). Females outnumber males by a ratio of approximately 2 to 1.[1] The predominant site of occurrence is the lower extremities (44%), which is also the site most frequently affected in young adult patients. The second most common site of occurrence for ASPS lesions is the head and neck (27%), which happens to be the most common site of occurrence of ASPS in infants and children.[1,2]

Head and neck ASPS is principally a disease of the orbit (41% of head and neck cases of ASPS) and tongue (25% of head and neck cases of ASPS), with lesser numbers of tumors involving the neck, temporal region, parotid region, nasoethmoid region, and jugulodigastric region.[18-22] When ASPS involves the orbital region, the affected patients are older as a group than those who develop ASPS at other head and neck sites.[20-22]

An ASPS of the head and neck typically presents as a slow-growing, painless mass. It often manifests with functional impairment related to the primary lesion (including proptosis, eyelid swelling, blindness, fixation of the globe, and difficulty speaking). These head and neck lesions therefore present at a lower stage (smaller primary lesion and fewer initial metastases) than do extremity lesions (which frequently present by way of symptomatic metastases).[1,5]

The most noteworthy radiographic finding for this lesion is its rich vascularity as seen on angiography. This may, in some patients, be confirmed on physical examination by demonstration of an audible bruit in the vicinity of the tumor.[23-25] As a radiographic differential diagnostic problem, however, this clinical finding, coupled with shunting seen on an arteriogram, may lead to confusion of ASPS with a benign arteriovenous malformation. It has been noted that ASPS shows a high signal intensity on T1-weighted and T2-weighted magnetic resonance images, an unusual finding among soft tissue sarcomas (most of which are isointense to muscle on T1-weighted magnetic resonance images).[25]

PATHOLOGIC FEATURES

Grossly, ASPS tends to be a poorly circumscribed lesion with friable, hemorrhagic, yellow-red cut surfaces. Careful scrutiny often reveals that the periphery of the tumor is encircled by large blood vessels. Microscopically, ASPS consists of a proliferation of large polyclonal cells with granular eosinophilic cytoplasm that are rather similar to one another in terms of cell size; mitotic figures are rare, and each tumor cell contains one or more vesicular nuclei with small nucleoli (see Figure, A,B). At low (scanning) power, the cells are organized in nests that are separated from one another by thin vascular septa (see Figure, A). Toward the center of many of these nests, the cells lose their cohesiveness and become necrotic, yielding an alveolar appearance. Often, lesions seen in

PATHOLOGIC DIFFERENTIAL DIAGNOSIS OF ALVEOLAR SOFT PART SARCOMA

Alveolar soft part sarcoma
Renal cell carcinoma
Paraganglioma
Granular cell tumor
Alveolar rhabdomyosarcoma
Angiosarcoma
Malignant melanoma

children will exhibit more of a sheetlike appearance. The small nests (alveoli) of polygonal tumor cells are in turn grouped into larger masses by thicker fibrous septa. At the margin of the tumor there are usually numerous dilated veins that are frequently the site of identifiable foci of angiolymphatic invasion.

One particularly distinctive feature of ASPS — found in three quarters or more of these tumors — is the finding of periodic acid–Schiff (PAS)–positive, diastase-resistant rhomboid and rod-shaped crystals in a sheaflike configuration within the cytoplasm of tumor cells (see Figure, C). Lesions that fail to demonstrate these well-formed crystals will usually show PAS-positive granular material in their cytoplasm that is thought to be a crystal precursor.

Immunohistochemically, this tumor most consistently marks with antibodies against vimentin and desmin[8,9,12]; there is some debate as to whether these tumors are positive for actin, myosin, S-100 protein, or NSE.[6,8-13] There is general agreement that ASPS does not express myoglobin, cytokeratin, epithelial membrane antigen, neural filaments, glial fibrillary acidic protein, serotonins, synaptophysins, met-enkephalins, or leu-enkephalins.[9-13]

Ultrastructurally, ASPS is characterized by groups of neoplastic cells separated from the intervening vascular channels by an incomplete basal lamina with linear densities along the inner surface of the cell membranes where contact with the basal lamina occurs. True myoepithelial cells are absent, but intervening spindle cells can be seen. Mature, well-formed cell junctions are absent, with total loss of cell cohesion seen near the center of each nest. The cells themselves contain an ample Golgi complex that is invariably associated with 120-nm dense granules inserted between the Golgi saccules. Larger granules can be seen at the periphery of the Golgi complex. There are numerous mitochondria with increased numbers of cristae, and there is a large amount of smooth and granular endoplasmic reticulum, which on occasion appears to anastomose. Clusters of lipid droplets and glycogen pools have also been identified.[5,6,10,26]

The most characteristic ultrastructural feature of ASPS is the presence of membrane-bound rhomboid, rod-shaped, and spicular cytoplasmic crystals.[27] The crystals consist of filamentous structures, made up of 2 globular substances, each with a diameter of 60 Å, arranged in a lattice structure with a periodicity of 100 Å. The large granules and crystalloids of ASPS are different from the granules seen in carotid body tumor, malignant melanoma, and granular cell tumor.[26]

The light microscopic pathologic differential diagnosis is shown in the Table. Renal cell carcinomas lack PAS-positive crystalline material, and may be cytokeratin-positive. Granular cell tumors and paragangliomas lack glycogen. Granular cell tumor cells have (as their name suggests) a more granular cytoplasm, with less well-defined cell borders, and less stromal vascularity. They exhibit strong nuclear S-100 protein staining. Paragangliomas often contain NSE- and S-100 protein–positive cells. Rhabdomyosarcomas are desmin- and actin-positive, while angiosarcomas are CD31- and CD34-positive and melanomas are S-100 protein–positive.[1,12]

TREATMENT

The mainstay of therapy for ASPS continues to be radical surgical excision of the tumor with an attempt at obtaining tumor-free margins.[1,20,21] While some clinicians have tried to improve patient survival by the addition of adjuvant irradiation and chemotherapy, no apparent benefit has been demonstrated to date for such adjuvant therapy.[19]

COURSE OF DISEASE

Viewed over the short term, the natural history of ASPS (taking tumors arising in all body sites) is a deceptively indolent one, in that 3- to 5-year survival is on the order of 65% or so. When these same patients are followed for an additional decade or so, however, it becomes apparent that ASPS is one of those tumor types that is prone to late recurrences and metastases; 10-year survival drops to 38%, while the 20-year survival is a dismal 15%.[19] Some patients have developed metastatic deposits decades after an initial surgical excision.

It appears that lesions of the orbit are associated with a longer disease-free interval than are nonorbital head and neck lesions.[20,21] Overall, for all sites, it appears as though the disease-free survival for children is improved over that seen in adult patients with ASPS; it is not clear whether this is a result of the greater incidence of head and neck lesions (which may present at an earlier stage and so may be more amenable to successful excision) in these pediatric patients.

When metastases occur, they most often are found in the lung, brain, and skeleton. In view of the rich vascularity of ASPS, it is perhaps not surprising that many metastatic deposits are identified either at or soon after initial diagnosis. It has been suggested that ASPS is unique among soft tissue sarcomas in that it is more likely than any other sarcoma to initially declare itself by virtue of symptoms produced by cerebral metastases.[1] Nodal metastases are infrequent.

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SIGNIFICANCE OF DNA PLOIDY AND CELL PROLIFERATION IN JUVENILE RESPIRATORY PAPILLOMATOSIS

YORAM STERN, MD
PAUL E. HURTUBISE, PHD
ROBIN T. COTTON, MD
CINCINNATI, OHIO

The clinical course of recurrent respiratory papillomatosis (RRP) in children is variable and unpredictable. At present there is no way to identify patients at risk for aggressive disease. The objective of this study was to evaluate whether DNA ploidy and cell proliferation analyses can predict the clinical course in children with RRP. Two different methods of estimating proliferation activity were compared. Nonembedded papilloma biopsy specimens from 18 pediatric patients were analyzed by flow cytometry providing DNA content with cell cycle analysis. The expression of the proliferative marker Ki-67 in papilloma tissue was quantified by immunohistochemistry. The patients were prospectively observed for 12 to 18 months. DNA content analysis and Ki-67 expression were compared to clinical information regarding number of disease sites, distal tracheobronchial spread, number of recurrences, need for tracheostomy, and disease remission. High S-phase fraction, proliferative index, and Ki-67 expression correlated with an aggressive clinical course. DNA ploidy analysis and immunodetection of proliferative markers may assist in predicting prognosis in children with RRP.

KEY WORDS — children, DNA content, Ki-67, papilloma.

INTRODUCTION

Recurrent respiratory papillomatosis (RRP) is the most common benign neoplasm of the larynx in children.[1] Although histologically a benign neoplasm, by virtue of obstruction of the airway, RRP may be a serious and potentially life-threatening disease. Characteristically, the clinical course of juvenile-onset RRP is unpredictable; some patients have a solitary lesion that responds to a few treatments, while others have lesions that recur chronically, arise from multiple sites, and distally spread to the tracheobronchial tree, requiring multiple surgical procedures. One of the more perplexing issues regarding RRP that remains unresolved is how to predict the course and severity of disease in an individual patient. Despite attempts to link the clinical course and prognosis of RRP with the endoscopic findings at the initial endoscopy,[2] histologic findings,[3-5] human papillomavirus (HPV) subtype,[6] and viral coinfections,[7] there is no way at present to identify those patients at risk for aggressive disease. There is growing evidence that cell proliferation has prognostic significance in malignant neoplasms.[8] Cell proliferation in tissues can be estimated by either flow cytofluorometry (FC), which evaluates cells in the S-phase of the cycle, or immunohistochemistry (IHC), which detects proteins present in cycling cells. The use of FC allows automatic quantification of the nuclear DNA content with cell cycle analysis of a large number of individual cells. Immunodetection of nuclear proteins in cycling cells allows not only quantification of proliferating cells, but also their precise localization. Ki-67 is a nuclear antigen that is expressed in all phases of the cell cycle[9] and is considered to be the best immunohistologic proliferation marker available.[10] Both FC and IHC are widely used to evaluate proliferating cells in malignant tumors.[11] However, these methods have been rarely used to analyze cell proliferation in benign lesions.

The objectives of the present study were to detect and quantify cell proliferation in respiratory papillomatosis and to estimate whether the proliferative activity correlates with the clinical course of the disease.

MATERIALS AND METHODS

PATIENTS

Pediatric patients (age less than 18 years) who underwent microlaryngoscopy and bronchoscopy for treatment of RRP between October 1995 and August 1996 were enrolled into the study. A biopsy specimen was obtained and processed by FC (nonembedded tissue) and IHC (formalin-fixed, paraffin-embedded tissue). The patients were then observed prospectively for at least 12 months and treated as neces-

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From the Department of Pediatric Otolaryngology and Maxillofacial Surgery, Children's Hospital Medical Center (Stern, Cotton) and the Department of Pathology and Laboratory Medicine, University of Cincinnati Medical Center (Hurtubise), University of Cincinnati College of Medicine, Cincinnati, Ohio.

Presented at the meeting of the American Laryngological Association, Palm Beach, Florida, May 9-10, 1998.

CORRESPONDENCE — Yoram Stern, MD, Dept of Pediatric Otolaryngology, Children's Hospital Medical Center, 3333 Burnet Ave, Cincinnati, OH 45229.

sary. Indications for operation were hoarseness, airway obstruction, or detection of gross respiratory papilloma by flexible laryngoscopy.

Sites of involvement (supraglottis, glottis, subglottis, trachea, bronchi, lungs, pharynx, and esophagus), number of recurrences, treatment modalities, and duration of clinically active disease were recorded. The average recurrence frequency for each patient was calculated from the patient's total number of surgical procedures and the time of active disease. Clinical remission was defined as complete absence of gross papillomas in at least 2 consecutive examinations over a minimum of 6 months.

FLOW CYTOFLUOROMETRY

Preparation of Nuclei for DNA Analysis. Nuclei from fresh biopsy specimens of papilloma tissue were isolated by a modification of the method originally described by Vindelon et al.[12] Briefly, the tissue was placed in a petri dish, minced finely with a razor blade, and forced through a fine wire mesh with a Teflon spatula. The resultant cell suspension was washed several times and filtered through 53-μm nylon mesh. The cells were then treated with a detergent-trypsin solution to release the nuclei. The concentration of the nuclei in the suspension was determined after it was filtered again through the nylon mesh. The nuclei suspension was treated with RNAse, stained with propidium iodide for 30 minutes, and analyzed for DNA content by flow cytometry.

Evaluation of DNA Content. The stained nuclei in suspension were analyzed on a Coulter XL-MLC flow cytometer with use of an argon laser (488 μm) to excite fluorescence of the propidium iodide intercalated into the DNA of the nuclei. Data on the measurements of DNA content of each nucleus by fluorescent intensity were collected in list mode for analysis by Multicycle Software (Phoenix Flow Systems, Inc, San Diego, Calif). This software is capable of debris modeling and sliced-nucleus correction when partial nuclei are detected.[13] The G0/G1 and G2/M peaks were assumed to be Gaussian curves and restrained to a fixed G1-to-G2 ratio of 1.97. Further determination of nuclei in the S-phase of the cell cycle was done by assuming a Gaussian-broadened distribution.[14] A nonlinear least-squares curve-fitting method was used to fit their Gaussian curves.[15] The S-phase was assumed to be asynchronous. Controls for each analysis included normal cells analyzed before and after each test sample to ensure accuracy of the test sample data. Additionally, internal controls for each of the control and test samples analyzed were performed with propidium iodide–stained chicken red blood cells that were nucleated.[16]

The DNA index (DI) was calculated by dividing the modal channel number of the peak with higher DNA content by that of the peak with lower DNA content. Aneuploidy was defined by a DNA index of more than 1.10. The S-phase fraction (SPF) and the proliferative index (PI = SPF + G2/M fraction) were calculated from DNA histograms by DNA cell cycle analysis software (Multicycle). Individual S-phase estimates were derived when the ploidy value and quality of the histogram were such that S-phases of the DNA-diploid and -aneuploid peaks did not completely overlap. Utilizing the DNA-aneuploid S-phase, when available, provides greatly superior prognostic value compared to the use of the average of the DNA-diploid and -aneuploid S-phases.[17] Therefore, we used the SPF and PI of the DNA-aneuploid cells for statistical analysis.

IMMUNOHISTOCHEMISTRY

The samples were fixed in formaldehyde and paraffin-embedded, and 4-μm sections were obtained. A section of each sample was stained by hematoxylin and eosin to confirm the diagnosis of respiratory papillomatosis. The other specimens were deparaffinized with xylenes and hydrated through graded alcohol. The sections were then incubated with the monoclonal antibody MIB1 (1:100; AMAC, Inc, Westbrook, Me) overnight at room temperature. Next, the slides were incubated with a biotinylated goat anti rabbit secondary antibody, followed by horseradish peroxidase–avidin, and the colorimetric reaction was visualized with diaminobenzidine and hydrogen peroxide by means of copper sulfate enhancement. Negative control sections were included. All slides were counterstained with hematoxylin. The ratio of Ki-67–positive cells was expressed as the percentage of at least 1,000 cells counted randomly at 400× magnification. Stained nuclei were regarded as positive, irrespective of staining intensity. Ki-67 staining in basal and parabasal cells was excluded, since Ki-67 staining in these cells is considered normal in epithelium.[18]

STATISTICAL ANALYSIS

Statistical analysis was done with SAS 6.08 Statistical Software (SAS Institute, Cary, NC) on a mainframe computer of the University of Cincinnati Computer Center. Kendall's correlation coefficient was used to calculate associations among the variables. The independent prognostic significance of SPF, DI, PI, and Ki-67 expression was evaluated by stepwise regression analysis. A p value of less than .05 was considered significant.

RESULTS

Nineteen patients were enrolled in the study. One

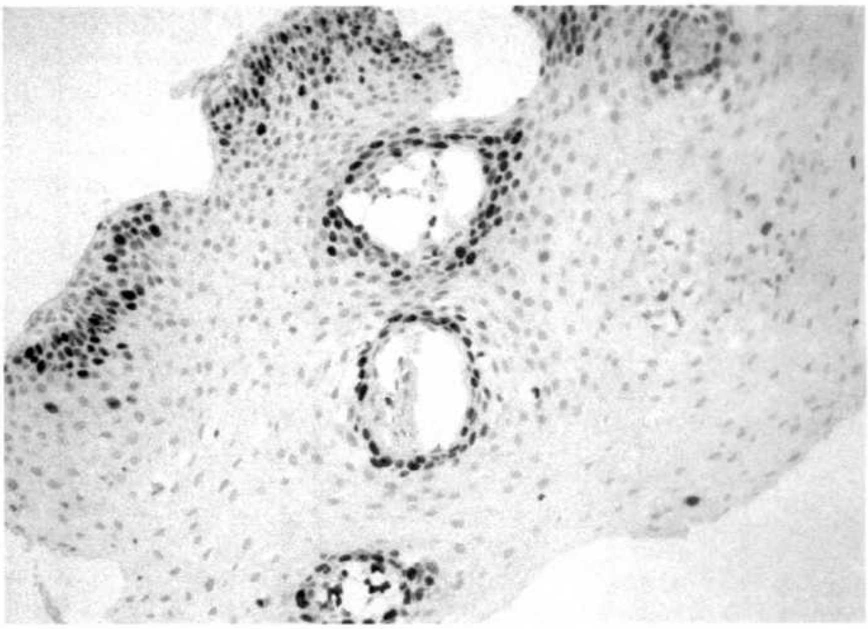

Fig 1. Immunoreactivity (brown staining) for Ki-67 in basal and parabasal cell layers with mild suprabasal staining (original ×50).

specimen was not suitable for FC; therefore, this patient was excluded from the study (N = 18). Age at time of entry into the study ranged from 1.5 to 18 years (mean ± SD, 6.2 ± 4.2 years), and the male-to-female ratio was 2.1 to 1. All patients had been followed for at least 12 months (mean 14.2 months). The number of disease sites that were observed during the study period in each patient ranged from 1 to 5 (mean ± SD, 3.1 ± 1.4), and the frequency of recurrences ranged from 0.17 to 1.58 procedures per month (mean ± SD, 0.63 ± 0.41). Seven patients had distal tracheal or bronchial spread of the papilloma. Three patients underwent tracheostomy prior to entry into the study. One patient required tracheostomy during the study period. All 4 patients remained tracheostomy-dependent at the end of the study period. Two patients (11.1%) had complete remission during the study period.

FLOW CYTOFLUOROMETRY

Ploidy. Seven samples had diploid DNA and 11 samples had aneuploid DNA (DI ≥ 1.10).

S-Phase. The SPF ranged from 2.2% to 36.4% (mean ± SD, 13.5% ± 10.1%).

Proliferative Index. The PI ranged from 3.0% to 38.9% (mean ± SD, 15.1% ± 10.3%).

IMMUNOHISTOCHEMISTRY

In all papilloma samples, many basal and parabasal

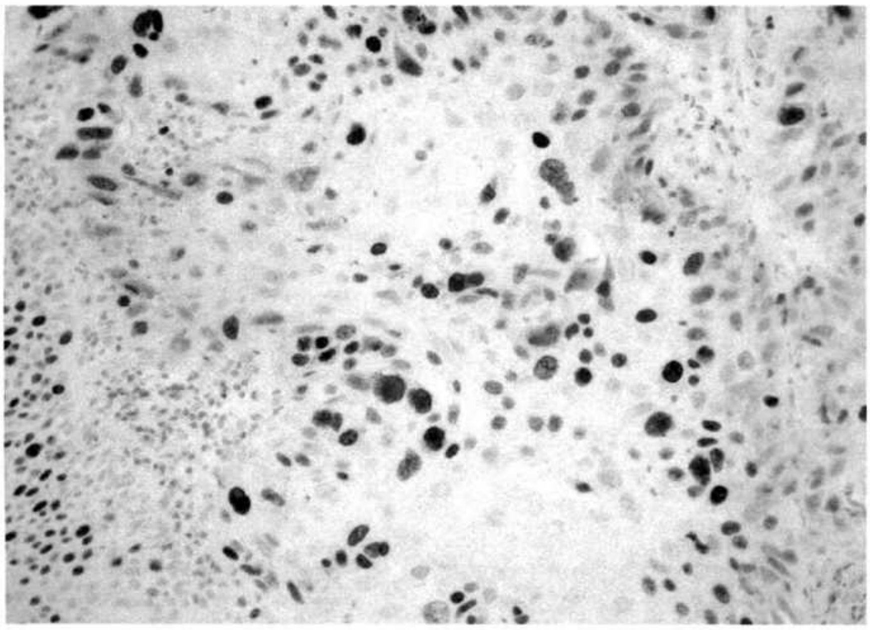

Fig 2. Immunoreactive nuclei scattered in suprabasal layers (original ×50).

SIGNIFICANT INDEPENDENT PROGNOSTIC FACTORS (P VALUES)

	No. of Disease Sites	*Recurrence Frequency*	*Tracheal or Bronchial Spread*	*Remission*
SPF	.006		.0299	
PI		.0001		
Ki-67				.0036*

SPF — S-phase fraction, PI — proliferative index.
*Inverse correlation.

cells were strongly positive for Ki-67 (Fig 1). The Ki-67 expression in the suprabasal layers ranged from 0% to 80% (mean ± SD, 46.4% ± 24.6%; Fig 2).

CORRELATION BETWEEN INDEPENDENT VARIABLES

The SPF correlated significantly with DI, PI, and Ki-67 expression. The PI was significantly correlated to DI and Ki-67 expression.

CLINICOPATHOLOGIC CORRELATIONS

The significant independent prognostic factors for predicting the outcome are listed in the Table. The SPF was significantly higher in biopsy specimens from patients who had multiple disease sites and tracheal or bronchial spread. The PI was significantly higher in biopsy specimens from patients who had frequent recurrences. The Ki-67 expression in biopsy specimens from patients who underwent clinical remission was significantly lower.

DISCUSSION

The results of the present study indicate that FC and IHC are both effective methods for evaluating cell proliferation in respiratory papilloma. The number and quantity of cells recovered after mechanical dissection was sufficient to quantify the DNA nuclear content by FC in most papilloma samples (18/19). Until recently, the use of Ki-67 was limited to frozen fresh tissue. However, paraffin-embedded tissues are easier to manage than frozen preparations, and their epithelial morphologic characteristics are better presented. A new monoclonal antibody, MIB1, enabled detection of the antigen also in formalin-fixed, paraffin-embedded tissues.[19] We therefore used this antibody for IHC labeling and were able to obtain a well-defined nuclear positivity.

Our primary result suggests that cell proliferation is increased in more aggressive RRP and may participate in the pathogenesis of this disorder. The underlying mechanisms for the abnormal proliferation of respiratory epithelial cells leading to the formation of papillomas are unclear. Of the many proteins essential for replication of viral DNA, HPVs encode only the replication origin recognition proteins E1 and E2.[20,21] Therefore, replication of papillomaviral DNA must rely heavily on the host DNA replication machinery. Further, in situ hybridization of patient papilloma biopsy specimens reveals that productive HPV DNA amplification takes place only in the upper spinous layers that have long since exited from the cell cycle.[22] These differentiating cells normally do not replicate their chromosomal DNA and presumably no longer transcribe those genes essential for DNA synthesis. These observations imply that HPV must have a mechanism that reactivates the necessary host proliferation genes to facilitate its own DNA replication, leading to abnormal cell proliferation. It has been suggested that HPV may deregulate the cell cycle and increase epithelial cell proliferation by regenerating and/or degrading tumor suppressor gene products and thus preventing them from functioning as regulators of cell growth.[23,24]

In the current study, SPF, PI, and Ki-67 expression were significantly higher in biopsy samples of patients who had a more aggressive clinical course. Therefore, nuclear DNA analysis and immunodetection of nuclear proteins that are present in cycling cells (eg, Ki-67) may serve as useful prognostic factors. These methods can also assist in monitoring adjuvant therapy, such as interferon treatment, by evaluating cell proliferation during the course of treatment. Therefore, it may be possible to provide additional prognostic indicators for determining a rational therapeutic approach.

DNA analysis during different clinical stages of the disease, and further, larger prospective studies and other methods of evaluating cell proliferation, are needed to assess the diagnostic and clinical value of our primary results. Clarification of the role of cell proliferation in establishing the severity of RRP will improve our knowledge of the pathogenesis of the disease, and may provide a prognostic indicator relevant to the course and management of juvenile respiratory papillomatosis.

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RESULTS OF NEAR-TOTAL LARYNGECTOMY

BRUCE W. PEARSON, MD
JACKSONVILLE, FLORIDA

LAWRENCE W. DESANTO, MD
SCOTTSDALE, ARIZONA

KERRY D. OLSEN, MD
ROCHESTER, MINNESOTA

JOHN R. SALASSA, MD
JACKSONVILLE, FLORIDA

The clinical records of 225 patients undergoing primary or salvage near-total laryngectomy (NTL) for laryngeal and pyriform cancer were analyzed for local control and morbidity. If the primary cancer was laryngeal in origin, patients underwent a simple NTL; if it was pyriform, a minor modification called near-total laryngopharyngectomy (NTLP) was used. When NTLP was extended to include necessary portions of the tongue base or posterior pharyngeal wall, pharyngeal reconstructions were added. The principal outcomes studied were 1) 5-year local control of the primary cancer, 2) achievement of lung-powered shunt speech, and 3) incidence of aspiration. The local control of cancer was similar to that expected with total laryngectomy or laryngopharyngectomy. Conversational voice was achieved in 85% of patients surviving beyond 1 year. Some patients required additional surgery for voice — usually endoscopic dilation. Aspiration was absent if primary healing was achieved. It was troublesome in wound breakdown if the shunt was directly affected. Secondary anti-aspiration procedures were required in 9% of our patients — usually preserving shunt speech.

KEY WORDS — laryngectomy, near-total laryngectomy, pharyngectomy.

INTRODUCTION

Near-total laryngectomy (NTL) was first described as an "extended hemilaryngectomy" in 1979,[1] and under the name "subtotal laryngectomy"[2] in 1980. "Extended hemilaryngectomy" generated confusion by inviting comparison with the classic conservation operations, such as vertical partial laryngectomy or supraglottic laryngectomy. These were for T1 and T2 glottic and supraglottic lesions, not T3 and T4 cancers. "Subtotal laryngectomy" was an unsatisfactory term, too, because the supracricoid partial laryngectomies, such as that with cricohyoidopexy[3] or that with cricohyoidoepiglottopexy,[4] began to be called by this name. "Near-total" seemed the most suitable adjective, and it has completely replaced "subtotal" in notes and discussions at our own institution, notwithstanding the fact that others have used it for cricohyoidopexy or cricohyoidoepiglottopexy,[5] or for a fronto-anterior partial laryngectomy with a sliding epiglottoplasty repair.[6]

We do not classify NTL with the conservation operations, because neither the voice nor the airway is "conserved." For years, "conservation" operations included cordectomy, the vertical partial laryngectomies, hemilaryngectomy, and supraglottic laryngectomy. The cancer was removed, a glottic voice remained, and respiration continued through the nasal passages. A permanent tracheostomy was not required. To these were added Ogura's partial laryngopharyngectomy[7] and the supracricoid partial laryngectomies with cricohyoidopexy or cricohyoidoepiglottopexy. They also met these criteria.

Near-total laryngectomy resects a much larger specimen. It produces shunt (fistula) voice. It results in a permanent stoma for respiration. It is only indicated, in fact, when the true conservation operations are inadequate (for tumor removal[8]).

Near-total laryngectomy produces voice by maintaining the natural connection between the trachea and the pharynx. This is its functional advantage over total laryngectomy — and, at the same time, its disadvantage. Total laryngectomy, by completely separating the airway and the food-way, gave optimum protection of the lungs. Nowadays, the esophagus and trachea are commonly reconnected with a tracheoesophageal puncture (TEP)[9] when a total laryngectomy is performed. Therefore, the communicative disadvantage of a total laryngectomy has been diminished — and so has its pulmonary asset.

At the present time, there are probably only 2 advantages to NTL over a total laryngectomy. First, voice persists, without the need to continually purchase and replace synthetic prostheses.[10] Second, the air charge enters the pharynx directly; it does not have to start as a bolus in the esophagus. Therefore, voice is independent of the vagaries of cricopha-

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From the Departments of Otorhinolaryngology, Mayo Clinic Jacksonville (Pearson, Salassa), Mayo Clinic Scottsdale (DeSanto), and Mayo Clinic Rochester (Olsen).

Presented at the meeting of the American Laryngological Association, Palm Beach, Florida, May 9-10, 1998.

CORRESPONDENCE — Bruce W. Pearson, MD, Dept of Otorhinolaryngology, E3 Davis Building, Mayo Clinic Jacksonville, 4500 San Pablo Rd, Jacksonville, FL 32224.

TABLE 1. NEAR-TOTAL LARYNGECTOMIES (N = 225)

	Stage						*Prior Irradiation*	
Site and Description	*T1*	*T2*	*T3*	*T4*	*Totals*		*No.*	*%*
Subglottic (+ glottic extension)			2			Subglottic 2	0	0
Glottic (+ subglottic extension)		5	38	1	44	Glottic 72	8	11.1
Transglottic (glottic + supraglottic extension)		1	23	4	28			
Supraglottic (+ glottic extension)		25	24	1	50	Supraglottic 74	13	17.6
Supraglottic-vallecula (+ tongue base and/or tonsil)		1	4	8	13			
Supraglottic (+ high pulmonary risk)		11			11			
Pyriform (medial + aryepiglottic fold)		4	11	3	18	Pyriform 75	6	8
Pyriform (lateral ± part of posterior pharyngeal wall)		9	33	15	57			
Cancer, but not squamous			2		2	Other 2	0	0

ryngeal dysfunction and the alterations in postcricoid anatomy that attend the larger laryngopharyngeal resections.[11]

Previous communications on NTL and near-total laryngopharyngectomy (NTLP) have focused on their indications (lateralized carcinomas pervading the paraglottic space) and/or the surgical management and technique.[12-14] This study is focused entirely on the outcome. Does NTL offer patients with T3 disease the same high local control rate as total laryngectomy (with or without partial pharyngectomy)? This would be an advantage over historical irradiation results. And does NTL truly succeed in preserving a lung-powered voice without a prosthesis? If so, it would be a biologic alternative to total laryngectomy and TEP.

PATIENT SELECTION

Near-total laryngectomy has been 1 option for treating cord-fixing lateralized laryngeal cancers at our institution for over 23 years. The first patient (included in this series) was operated on in 1974. It was not until she had been followed 4 years that the second case was performed (1978). The surgical techniques became stabilized by 1981.[2,15]

In February 1997, the 300th patient underwent NTL in the Mayo Clinic Department of Otorhinolaryngology. To prepare this report, we selected only patients with a minimum of 5 years of follow-up, resulting in 234 patients. Nine exclusions (4 unrelated early deaths, 5 lost to follow-up) reduced this number to 225, and these patients are the subjects of our study.

For a case to be included in this report, the following anatomic criteria had to be satisfied. The whole larynx (and 1 pyriform sinus), from tongue to trachea, had to be resected except for an uninvolved strip, posterolaterally, on the side opposite the cancer. The entire paraglottic space on the side of the cancer had to be included within the resection en bloc. The uninvolved strip preserved the mucosal connection between the trachea and the remaining pyriform sinus to permit lung-powered shunt speech. Innervated muscular elements, namely, the cricothyroid and portions of the good-side intrinsic glottic musculature, had to be preserved for the prevention of aspiration. The resection of the cancer and the construction of the speaking shunt were 1 operation. No stent was required in the postoperative period to maintain the fistula, and no prosthesis was subsequently required to stent or valve the speaking shunt.

METHODS

The operative notes on all Mayo patients undergoing "laryngectomy" during the study period were reviewed. Patients who did not undergo the NTL technique as previously defined, such as 6 "neoglottic fistula" patients from the late 1970s, were excluded. The pathology records of those patients positively identified by procedure were reviewed to confirm the diagnosis of laryngeal or pharyngeal cancer. Eleven patients underwent NTL for nonmalignant indications: intractable aspiration from skull base surgery and lower cranial nerve disease, or voice-preserving laryngeal closure with pharyngectomy and pharyngeal reconstruction. These were excluded from this study. Information extracted from confirmed patient clinical records was entered into a spreadsheet program (Excel 97) and analyzed.

OBSERVATIONS

Between 1978 and 1992, 233 patients underwent NTL for laryngeal or pharyngeal cancer at all 3 Mayo Clinic sites. The index case of 1974 brings the study population to 234. One hundred ninety-three patients

TABLE 2. NEAR-TOTAL LARYNGECTOMY PATIENT CHARACTERISTICS (N = 225)

Age	*Male*	*Female*
29-40	2	4
41-50	9	6
51-60	44	9
61-70	75	22
71-80	43	4
81-91	6	1
Total	179	46

were operated on in Rochester, Minnesota, and (between 1974 and 1992) 41 received their surgery at the new Mayo Clinic practices in Jacksonville, Florida, and Scottsdale, Arizona. There were 4 early postoperative deaths unrelated either to the cancer or to the NTL. An additional 5 patients have been lost to follow-up. Therefore, 225 cases of NTL for laryngeal or pharyngeal cancer were available to form the subject of this report.

Table 1 indicates the site and stage of the primary cancers, as well as the frequency of prior irradiation. Eleven percent of the 225 patients in this series had undergone prior irradiation and therefore received an NTL for surgical salvage. While most of the glottic (66 of 72) and pyriform (62 of 75) patients were advanced-stage T3 or T4, the series includes 37 supraglottic lesions of a low stage, ie, T2. At least 11 of these were oncologically suitable for a supraglottic laryngectomy; NTL was selected because of advanced lung disease, age, etc, that rendered them physiologically unsuitable for supraglottic laryngectomy.[16] The adverse biologic potential of tumors in this series can be appreciated by noting that there are 32 T4 lesions of various sites, and 75 cancers of the pyriform sinus.

The age and sex distribution of the patients are detailed in Table 2. The predominant age group was 61 to 70, and 24% of the patients in this series were over the age of 70.

TABLE 4. NEAR-TOTAL LARYNGECTOMIES WITH LOCAL RECURRENCES IN SHUNT (N = 8/225)

Cases	*Site*	*Time to Recurrence (mo)**	*Previous Irradiation†*	*Salvaged§*
1	Glottic	37	No	Yes
3	Supraglottic	9	Yes	No
		45	Yes	No
		81	No	Yes
1	Tongue base–vallecula	8	Yes	No
3	Pyriform sinus	14	Yes	No
		21	Yes (for unknown primary)	No
		31	No	No

*Average 31 months.
†63% had previous irradiation (5/8).
§25% were salvaged (2/8).

One hundred forty-eight of our patients underwent "simple" NTL (Table 3). Their cancer usually arose within the larynx, and the narrow laryngeal remnant usually required augmentation of its diameter with a small myomucosal pull-down flap from the pharynx. Fifty-eight patients with pyriform cancer underwent NTLP, in which more pharynx was excised posteriorly and less larynx anteriorly. In these patients, one loses too much pharynx to donate a flap to the laryngeal remnant. However, the entire (vertical) hemilarynx — enough to make the vocal shunt without augmentation — is preserved. Nine patients underwent "extended" NTLP, in which the pharyngeal resection was so extensive, flap reconstruction of the pharynx was required for deglutition. In 3 additional patients the flap was free jejunum[2] or radial forearm skin. The 5 patients designated "NTL plus" underwent simple NTL and extensive resection of the tongue base–tonsil region. A flap was required for the oropharyngeal repair. Finally, there were 2 cases difficult to classify, called "atypical" NTLs. In 1, the entire shunt came from a posterior laryngeal remnant, and in the other, the tip of the epiglottis was preserved.

TABLE 3. TYPES OF NEAR-TOTAL LARYNGECTOMY (N = 225)

	Primary Tumor					
Type of Operation	*Subglottic*	*Glottic*	*Supraglottic*	*Pyriform*	*Nonsquamous*	*Total*
NTL	2	72	66	6	2	148
NTLP				58		58
NTLP with flap*			1	8		9
NTLP with free flap*				3		3
NTL plus with flap†			5			5
Atypical "NTL"§			2			2

*"Extended" near-total laryngopharyngectomy (NTLP; excision of pharyngeal extension required flap for reconstruction).
†Plus excision of tongue base–tonsil tissue that required flap for reconstruction.
§Near-total laryngectomy (NTL), but in 1 case, epiglottic tip was preserved, and in another case, opposite-side posterior larynx contributed to shunt construction.

TABLE 5. NEAR-TOTAL LARYNGECTOMIES WITH ASPIRATION SUFFICIENT TO WARRANT REVISION

	No.	*%*
Glottic	3/44	7
Supraglottic	4/74	5
Transglottic	4/28	14
Subglottic	0/2	0
Pyriform sinus	8/75	11
Nonsquamous	1/2	50
Total	20/225	8.9

Is NTL effective in achieving local control of the disease? Obviously, we would not expect NTL to alter treatment or control in the neck or the lungs, but we would expect local control rates comparable to those of total laryngectomy. Table 4 shows the incidence of local recurrence in the 225 patients followed up longer than 5 years.

Eight local recurrences appeared in the laryngeal shunt. One appeared 6½ years after surgery and may represent a second primary. Five developed in patients whose NTL or NTLP was for surgical salvage of irradiation failure. Two had radiorecurrent supraglottic cancer, 2 pyriform, and 1 tongue base–vallecula. In retrospect, NTL and NTLP should probably not have been offered to these patients. Of the 8 local (shunt) recurrences, only 2 were salvaged by total laryngectomy. Five patients went on to die of their disease, and 1 of other causes.

Seven additional patients, classified as having local recurrences in this series, developed cervical visceral cancers that were not associated with the laryngeal shunt. Two were stomal recurrences. Five occurred at sites anatomically distant from the laryngeal shunt (nasopharynx, tongue, and esophagus). Thus, in a series that makes no exclusions for neck disease, and includes irradiation salvage patients, T4 cancers, and patients with pyriform primaries, the *total* "local" recurrence rate after NTL, NTLP, or "extended" NTLP is 7%. Only 4% could be tied to preservation of a speaking shunt. And this figure could have been pushed under 2%, had we clearly understood that in patients with supraglottic or pyriform cancer, failed radiotherapy is a *contraindication* to NTL or NTLP.

Local complications of NTL fall into 2 categories. The first is the inability of the patient to achieve conversational voice, and the second is aspiration through the shunt. Table 5 indicates the number of patients who experienced sufficient aspiration to warrant revision surgery. This was 9% in our series. This figure includes our earliest cases, operations done by 10 different consultants, and a number of chief resident cases assisted by these consultants.

TABLE 6. NEAR-TOTAL LARYNGECTOMY VOICE RESULTS

	Able to Talk With Shunt		*Unable to Talk With Shunt*	
	No.	*%*	*No.*	*%*
Glottic	40/44	91	4/44	9
Supraglottic	59/74	80	15/74	20
Transglottic	26/28	93	2/28	7
Subglottic and other	3/4	75	1/4	25
Pyriform sinus	64/75	85	11/75	15
Total	192/225	85	33/225	15

The number of patients *un*able to talk with their shunt is provided in Table 6. Some of these patients *could* demonstrate shunt speech, but not without prompting. For conversational speech, they were reliant on esophageal speech, electromechanical devices, or writing. The overall percentage of patients *unable* to talk with their shunt was 15%. The percentage *able* to talk with their shunt was 85%. Speech, once acquired, was generally maintained.

DISCUSSION

During the period under study, our indications for NTL have been as follows.

1. Invasive lateralized vocal cord cancer in which conventional vertical partial laryngectomy is contraindicated by vocal cord fixation or limited mobility with subglottic extension. Classic or extended hemilaryngectomy procedures in these tumors have discouraging local failure rates,[17] and when radiotherapy is selected and fails, these patients are probably no longer candidates for NTL.

2. Lateralized transglottic carcinoma with fixation. Serial section studies indicate that 30% to 50% of T3 lesions are understaged.[18] Near-total laryngectomy is an operation that will accommodate this possibility. It encompasses lesions with thyroid cartilage and even cricoid cartilage invasion. It allows one to recognize an error in staging during the course of a resection.

3. Epiglottic and false vocal cord cancer beyond the scope of a supraglottic laryngectomy. In supraglottic cancer, total laryngectomy is rarely indicated in our practice, since NTL will address the discovery of glottic, medial pyriform, or tongue base extension. With an NTL, the patient can still manage a lung-powered voice, and the problem of postoperative aspiration seen in extended supraglottic partial laryngectomy is largely eliminated.

4. T2 supraglottic cancer in a patient who is physiologically unable to tolerate a supraglottic laryngectomy[16] or a supracricoid laryngectomy with crico-

hyoidopexy.

5. Aryepiglottic fold cancer beyond the scope of Ogura's partial laryngopharyngectomy.

6. T2 or T3 pyriform cancer. Pyriform cancers that involve the apex of the pyriform sinus, invade cartilage, and/or fix the hemilarynx are candidates.[19] However, the presence of postcricoid or interarytenoid spread, anterior laryngeal spread to the opposite side (eg, within the thyroid cartilage), and posterior pharyngeal wall spread to the opposite pyriform sinus are important contraindications.

For these indications, NTL seems to offer a satisfactory local control rate and an 85% chance of voice restoration (which is comparable to TEP fistula speech). The TEP option is preserved in the 15% instance of failure. An intriguing question we have *not* answered here is whether a stoma, the principal drawback of NTL, can be averted.[20] In this regard, the most promising advance of which we are aware is minimally invasive transoral laser microresection. Steiner and others in Germany are able to achieve NTL resection endoscopically using this technology, and no reduction in local control is reported.[21,22] Following their excellent lead in our own practice, we find that minimally invasive transoral laser microresection *does* preserve sufficient cricoid integrity and extralaryngeal support that a stoma is *not* required.

In reviewing these data, we have paid particular attention to factors, if any, that might predict local recurrence. Our goal is to reduce this risk. Irradiation has little impact in diminishing the functional outcome of NTL when given *after* surgery, and histopathologic findings in the neck dissection specimen (like extracapsular invasion) often made *postoperative* radiotherapy advisable. Therefore, numerous patients included in the results reported here received postoperative radiotherapy, for neck indications, and it could certainly be maintained this helped the local result. Radiotherapy was never used to finesse local margins, however. We would abandon NTL in this series unless negative intraoperative frozen section margins could be obtained. With these understandings, if NTLs were done for glottic or transglottic carcinoma, the local recurrence related to the shunt was 2%. Similarly, supraglottic and pyriform carcinoma had a shunt recurrence rate of 2.4% *if they were previously untreated.* However, NTL for supraglottic or pyriform carcinoma *after failed radiotherapy* was associated with a shunt recurrence rate of 20%, and surgical salvage was 0%. We offer this as an argument for restricting NTL to previously untreated patients. Near-total laryngectomy is not a salvage operation.

Significant aspiration occurred in 9% of the patients in this series. However, more than half of the patients with significant aspiration underwent successful revision surgery yet maintained their shunt speech. The only NTLs that required completion laryngectomy for aspiration were those that were functionally adynamic. An adynamic shunt is avoided by guarding against recurrent laryngeal nerve injury on the noninvolved side or excessive cauterization of the muscles in the laryngeal remnant.

The rate of speech acquisition after NTL was 85%. Since most patients with supraglottic cancer who are not candidates for supraglottic laryngectomy can undergo an NTL, there should be very few patients treated by surgery for supraglottic laryngeal cancer who do not achieve a laryngeal voice. Esophageal speech, TEP, and electromechanical aids all remain options for NTL patients who do not achieve shunt speech, provided the patient's spirit and motivation are sustained.

CONCLUSIONS

This study presents data on 225 patients undergoing NTL for intermediate and advanced cancer of the larynx or hypopharynx that would otherwise have been subjected to total laryngectomy techniques. The local recurrence rate was 7%. Irradiation failure should probably be regarded as a relative contraindication to NTL. Near-total laryngectomy is an effective treatment option for appropriately selected patients, and satisfactory functional results have been achieved.

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HUMAN VOCALIS CONTAINS DISTINCT SUPERIOR AND INFERIOR SUBCOMPARTMENTS: POSSIBLE CANDIDATES FOR THE TWO MASSES OF VOCAL FOLD VIBRATION

IRA SANDERS, MD
YINGSHI HAN, MD
SURINDER RAI, MD
HUGH F. BILLER, MD
NEW YORK, NEW YORK

It is not understood how different parts of the thyroarytenoid muscle contribute to vocal fold vibration. This study investigated the medial part of the thyroarytenoid muscle, the vocalis compartment, for anatomic differences that might suggest functionally distinct areas. Twenty human vocal folds were frontally sectioned and stained with hematoxylin and eosin. A single section from the middle of each vocal fold was magnified, and the muscle fascicles of the most superficial 25% of the vocalis compartment were then examined. In all 20 specimens the vocalis compartment could be separated into 2 plainly distinct subcompartments: the inferior vocalis compartment was composed of a single large muscle fascicle that contained densely packed muscle fibers of similar size; the superior vocalis compartment was composed of multiple small fascicles in which the muscle fibers were loosely arranged and varied greatly in size. On average, the inferior vocalis subcompartment composed 60% of the medial surface of the thyroarytenoid muscle. The superior subcompartment composed the remaining 40% of the medial surface, but also continued past the vocal ligament to make up the superior surface of the thyroarytenoid muscle. It is concluded that 2 distinct entities make up the vocalis compartment of the thyroarytenoid muscle. Their anatomy is so markedly different it suggests that they may function independently. One possibility is that they reflect the 2 masses observed in the superior and inferior aspects of the vocal fold during vibration.

KEY WORDS — compartments, larynx, thyroarytenoid muscle, vocal fold, vocalis muscle, voice.

INTRODUCTION

According to the aerodynamic-myoelastic theory as described by Muller (see Sonesson[1]) and Van den Berg,[2] vocal fold vibration is basically dependent on 3 factors: 1) subglottic air pressure, 2) extrinsic tension on the vocal fold (mostly supplied by the cricothyroid muscle), and 3) intrinsic tension within the thyroarytenoid (TA) muscle. At present, the intrinsic contribution of the TA muscle to the biomechanics of vocal fold vibration is probably the least understood. Numerous experiments with excised larynges have shown that the vocal folds can vibrate and produce sounds provided that they are placed together and that an adequate airflow is supplied.[3] In isolation, these experiments suggest that the TA muscle is a relatively unimportant, passive component of the vibrating vocal fold. However, human larynges can produce sounds with a variety of intensities, registers, and qualities that cannot be explained without an active TA muscle. In fact, electromyography of the TA muscle has shown that it is highly active during phonation,[4] but experiments have disagreed as to the effect this activity has on aspects of phonation such as control of pitch.[5]

Theoretically, TA activity can alter the biomechanics of the vibrating vocal fold in 4 ways: TA muscle fibers can shorten the vocal fold, change its shape, change the longitudinal stress in different areas of the muscle, and, possibly, insert into the mucosa and alter its stress directly. Most of these possibilities require that the TA not function as a homogeneous muscle but that it instead be capable of recruiting separate parts for various tasks. If so, these capabilities may be recognizable in specialized aspects of the TA muscle anatomy. Unfortunately, the TA is a complex 3-dimensional muscle that has frustrated classical anatomists.

The most basic anatomic or biomechanical distinction in the TA muscle is that of adduction by the lateral part versus phonation by the medial part. Wustrow[6] termed the medial part the portio thyrovocalis (vocalis) and the lateral part the portio thyromuscularis (muscularis). The muscularis portion originates from the muscular process of the arytenoid cartilage, while the vocalis portion originates from the vocal process. However, once they leave their origins, they are very difficult to trace, as they are not separated by a fascial barrier. This lack of classic criteria to differentiate and define the vocalis and muscularis portions has been a source of controversy.[7-9] Sones-

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From The Grabscheid Voice Center, Department of Otolaryngology, Mount Sinai Medical Center, New York, New York. This research was supported by grant R01 DC01764 from the National Institutes of Health/National Institute on Deafness and Other Communication Disorders.

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CORRESPONDENCE — Ira Sanders, MD, Box 1189, The Grabscheid Voice Center, Dept of Otolaryngology, Mount Sinai Medical Center, New York, NY 10029.

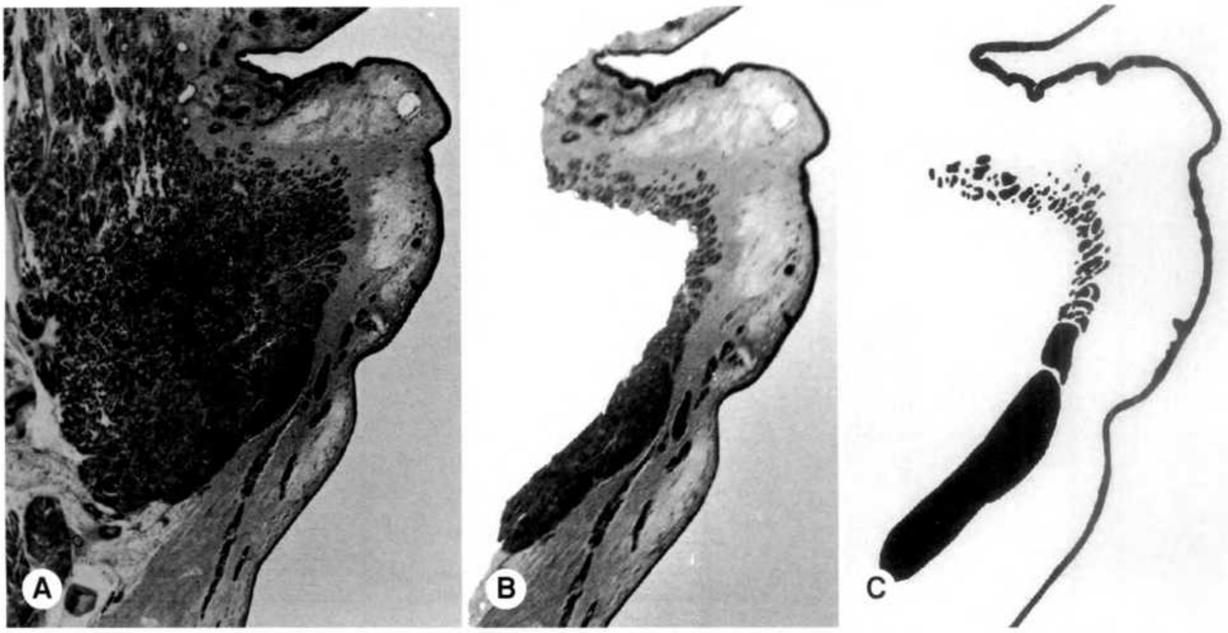

Fig 1. Method of preparing specimens for analysis. A) Frontal section through middle of membranous vocal fold (50 μm, H & E). B) Most of thyroarytenoid muscle is erased except for most superficial 25% of vocalis compartment. C) Muscle fascicles are reproduced in black ink to enhance contrast.

son[1] reexamined this question in great detail and concluded that the TA can only be considered to be a single muscle — a view that is widespread at present. More recently, muscle researchers have demonstrated that different parts of a single muscle do not have to be separated into grossly distinct bellies to have different functions.[10] Instead, some muscles are separable into functional entities called compartments, and the criteria used to delineate these compartments can be quite subtle, such as the percentage of fast and slow twitch muscle fibers. In fact, the distribution of fast and slow twitch muscle fibers has lately been shown to be dramatically different on either side of the human TA muscle. This allows the muscle to be roughly bisected into a fast-contracting lateral compartment, the muscularis, and a slow-contracting medial compartment, the vocalis.[11]

Assuming that the vocalis compartment exists, this study attempted to go a step further to find differences within it. Farnsworth[12] demonstrated that the superior and inferior aspects of the vocal fold vibrate out of sequence. This observation forms the basis for the 2-mass model of vocal fold vibration, the most accepted explanation of vocal fold vibration.[13] To date, there is no anatomic evidence for different masses within the human vocalis compartment, and the identification of candidates for these masses was a main goal of this study. Surprisingly, preliminary examination of human TA sections showed that the vocalis compartment of the muscle had grossly visible differences in the organization of its muscle fascicles. This study quantifies those differences in 20 human vocal folds and proposes that these differences divide the vocalis compartment into well-defined subcompartments (SCs). Further, these SCs appear to be plausible candidates for the anatomic substrate underlying the 2-mass model of vocal fold vibration.

METHODS

Twenty human larynges obtained from total laryngectomies were used in this study. These specimens were taken from the Goldman collection, which was established in 1960 at Mount Sinai Hospital. The records for age and sex were not available. Larynges were immersion-fixed in formalin after surgical excision and then embedded in celloidin. Each larynx was then frontally sectioned at 50 μm, and every 10th section was stained with hematoxylin and eosin. For this study a single section was chosen from the middle of the true vocal fold of every specimen. Then 1 vocal fold was selected for study, as the opposite fold was often involved with a tumor. The sections were projected at 21× magnification (Bausch and Lomb projection scope) onto paper, and the outline of the entire vocal fold and TA muscle was drawn. The muscle was then bisected into medial and lateral halves. The most superficial 25% of the muscle was reproduced on the paper by filling the outline of the muscle fascicles with dark ink (Fig 1). It was assumed that this percentage encompassed a large amount of that part of the TA muscle that participated in vibration. For the purpose of this study, this muscle mass was referred to as the vocalis compart-

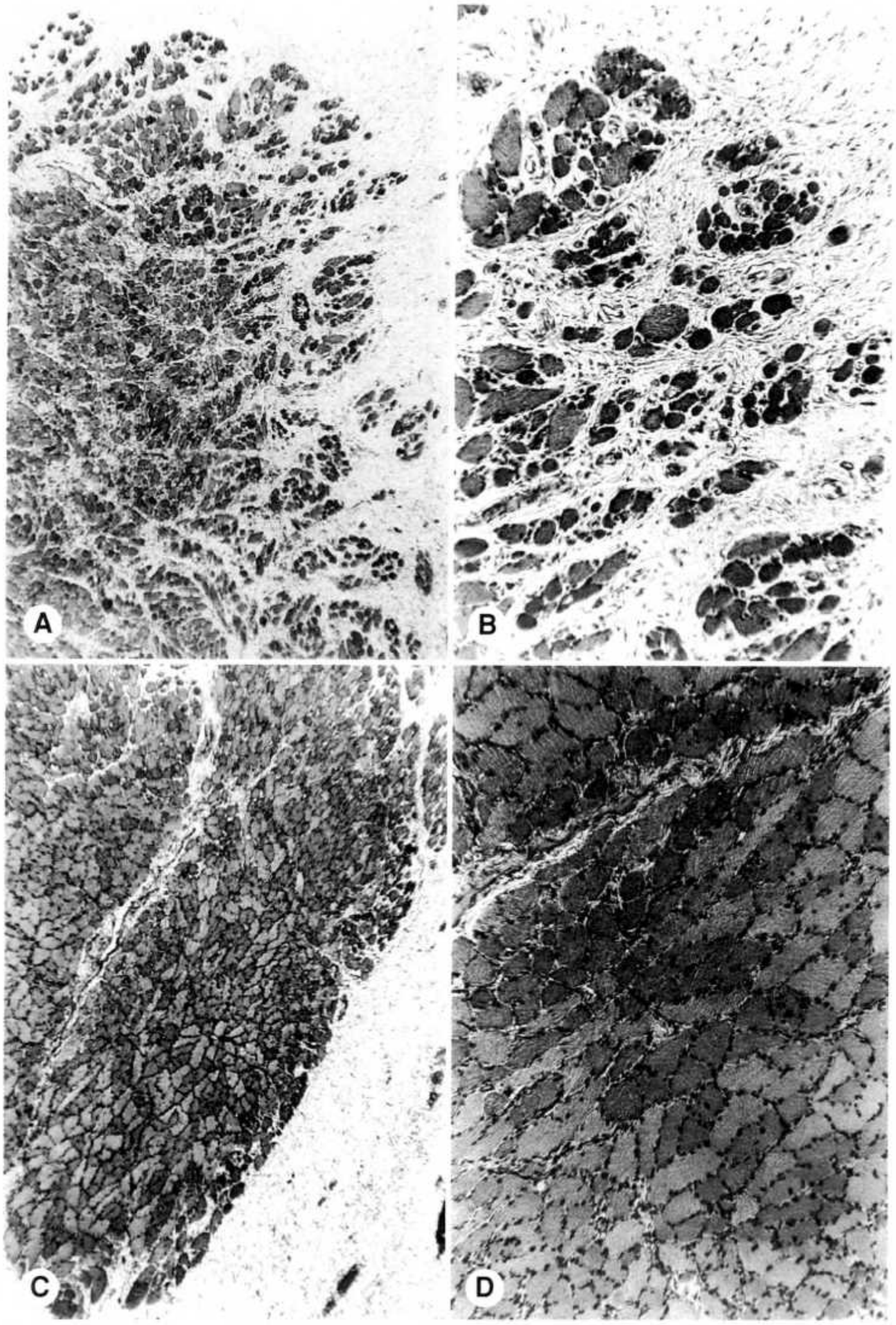

Fig 2. Photomicrographs of superior and inferior subcompartments. A) Superior subcompartment (original ×10). Muscle fascicles are separated by large amounts of connective tissue. B) Superior subcompartment (original ×25). Muscle fibers are loosely packed within fascicles and are of various sizes. C) Inferior subcompartment (original ×10). Muscle fascicle is homogeneous, with few fascial planes within it. D) Inferior subcompartment (original ×25). Note that muscle fibers are densely packed and similar in size.

ment. First, the entire length of the vocalis compartment was measured. Then the superior and medial surfaces of the vocalis compartment were measured. The point dividing the superior and medial surfaces was identified by drawing a line from the vocal ligament to the closest part of the muscle. The superior surface of the vocalis compartment was always found to be composed by part of the superior vocalis SC. In contrast, the medial surface of the vocalis compartment was made up partly by the inferior vocalis SC and partly by the superior vocalis SC. The lengths of the inferior and superior vocalis SCs on the medial surface of the muscle were measured and their percentages were calculated. The inferior vocalis SC was defined as the inferior muscle mass that was continuous (contained no significant amounts of internal connective tissue partitions), homogeneous (contained muscle fibers of relatively equal size), and densely packed (muscle fibers were approximated against one another without large amounts of connective tissue between them; Fig 2C,D). The superior vocalis SC was defined as the superior part of the muscle that was discontinuous (composed of muscle fascicle groups separated by large amounts

MEASUREMENT DATA IN MILLIMETERS

Sample No.	*Tot Length*	*Inf Voc*	*Sup Voc*	*Inf Length*	*Sup Length*
1	5.9	2.2	3.7	4.1	1.7
2	6.2	2.4	3.9	5.3	0.9
3	5.2	2.4	2.8	3.6	1.6
4	5.8	2.6	3.0	3.1	2.4
5	5.2	2.6	2.6	2.9	2.3
6	6.5	2.6	3.9	4.2	2.3
7	6.7	2.7	4.0	6.0	0.7
8	7.5	2.9	4.6	4.6	2.9
9	7.1	3.3	3.8	6.4	0.8
10	7.9	3.4	4.5	5.0	2.9
11	6.7	3.5	3.2	5.0	1.6
12	8.0	3.5	4.5	4.7	3.3
13	6.6	3.5	3.1	5.2	1.4
14	7.5	3.6	4.0	5.2	1.4
15	6.8	3.6	3.2	5.3	1.6
16	6.1	4.0	2.1	4.1	1.9
17	7.8	4.0	3.7	5.4	2.4
18	7.8	4.0	3.7	5.0	2.8
19	7.5	4.0	3.4	6.6	0.9
20	9.1	5.2	3.9	7.4	1.7
Average	6.9	3.3	3.6	5.0	1.9
SD	1.0	0.8	0.7	1.1	0.8

Tot length — total length of entire measured perimeter of thyroarytenoid (TA) muscle; inf voc — length of inferior vocalis subcompartment; sup voc — length of superior vocalis subcompartment, including both medial and superior perimeter; inf length — length of medial surface of TA muscle; sup length — length of superior surface of TA muscle.

of connective tissue), nonhomogeneous (muscle fascicles had large variations in individual muscle fiber size), and loosely packed (within a muscle fascicle the muscle fibers were separated by connective tissue; Fig 2A,B).

RESULTS

All 20 specimens demonstrated 2 distinct vocalis SCs: superior and inferior (see Table). The superior vocalis SC was composed of numerous small muscle fascicles in which the muscle fibers were loosely packed. In contrast, the inferior vocalis SC was in the form of a large single muscle fascicle within which the muscle fibers were densely packed. Although the exact appearance of each SC varied widely (Fig 3), all specimens adhered to the basic pattern. There were 15 right vocal folds and 5 left vocal folds, but they were all converted to the same orientation to allow the comparisons shown in Fig 3. The mean total length of the vocalis compartment was 6.87 mm (±1.02 mm). Out of this, the mean length of the superior vocalis SC was 3.58 mm (±65 mm; 52.4% of the vocalis compartment length) and that of the inferior vocalis SC was 3.30 mm (±0.76 mm; 47.7%). The length of the vocalis compartment below the vocal ligament (the medial surface of the vocalis compartment) was 4.95 mm (±1.1 mm; 72%), while the mean length above this point (the superior surface) was 1.9 mm (±0.76 mm; 27.6%). The inferior SC composed 60% of the medial surface of the vocalis compartment, with the remaining 40% being composed of the superior vocalis SC.

DISCUSSION

The results of this study show that the vocalis compartment of the human TA muscle is clearly separable into superior and inferior SCs. Although the overall shape and size of the vocalis compartment and its SCs varied greatly, all 20 specimens contained a basic pattern: an inferior SC that was composed of 1 large muscle fascicle in which the muscle fibers are of similar size and are densely packed, and a superior SC in which the fascicles are separated and the muscle fibers within these fascicles are loosely arranged and of different sizes (Figs 2 and 3). The presence of this pattern in all 20 specimens suggests that this is a universal pattern of the adult human vocal fold.

The impressive morphologic differences between the 2 SCs implies that they are designed for distinct functions. On the basis of our knowledge of general muscle morphology, it appears that the inferior SC is much like other muscles throughout the body. In contrast, it is the superior SC that appears unusual and, in some ways, unique. In most animals, including the chimpanzee,[14] the superior SC area is mostly composed of soft tissue. In these examples the medial TA muscle looks like the human inferior SC, a single densely packed fascicle. In the newborn human the superior SC area is also mostly soft tissue, with only a hint of the fasciculated muscle fibers seen in the adult.[15] Only the dog has a small muscle in the superior vocalis area that even approximates the morphology of the human superior SC.[16]

Another fact of comparative anatomy argues for a unique role for the superior SC. Of all species examined, only primates have been shown to have classic muscle spindles present within the laryngeal muscles.[17-19] The muscle spindle is a highly specialized muscle sensory organ that is used to sense muscle length and changes in muscle length.[20] Recently, the location of muscle spindles was mapped in 5 human TA muscles.[21] In that study it was found that muscle spindles are not randomly distributed within the human TA muscle; instead, 85% are concentrated in the superior SC area. These muscle spindles are distributed in an arc in the superficial layer of the muscle, in the same area in which the separate fascicles are seen in this study (Fig 4[21]). Therefore, the supe-

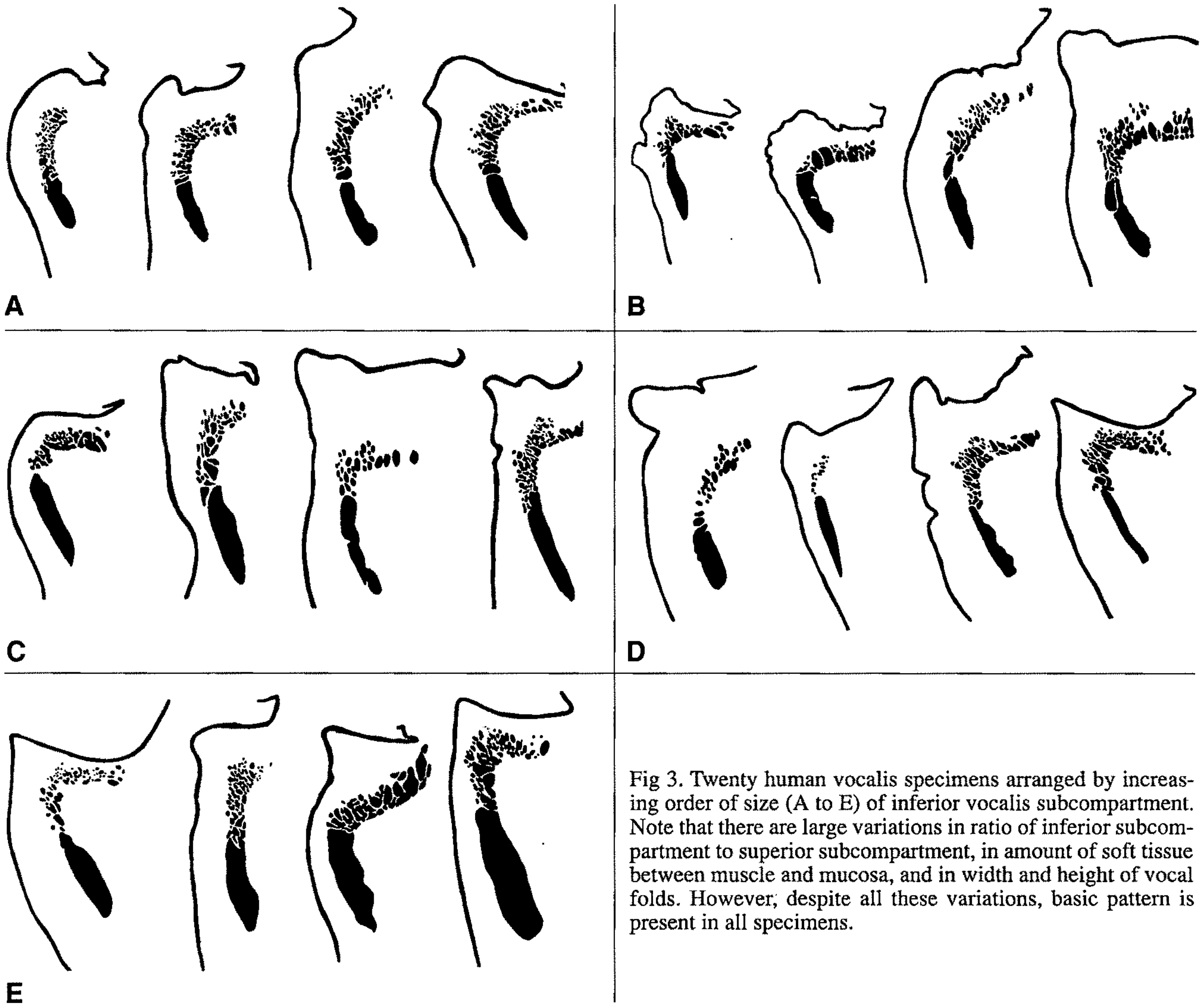

Fig 3. Twenty human vocalis specimens arranged by increasing order of size (A to E) of inferior vocalis subcompartment. Note that there are large variations in ratio of inferior subcompartment to superior subcompartment, in amount of soft tissue between muscle and mucosa, and in width and height of vocal folds. However, despite all these variations, basic pattern is present in all specimens.

rior SC appears to be singled out for highly specialized sensory control.

The above discussion indicates that the inferior SC is relatively similar in all animals, including humans. In contrast, a large and specialized superior SC appears to be evolutionarily unique and restricted to adult humans. These differences between the human and other species suggest that animal experiments on the vocal folds need to be interpreted with caution before generalizing their results to humans. These facts also imply that the human superior SC has evolved to play some unique role in speech, a skill restricted to humans. In addition, the morphology of the 2 SCs suggests that they may be controlled independently. It is even possible that the multiple fascicles of the superior SC may themselves be independently controlled.

The roles of the 2 SCs in phonation can only be speculated. As mentioned in the Introduction, the most basic theory of vocal fold vibration is the 2-mass model.[13] This model is based on observations of the vocal folds of a human male phonating in the modal register.[12] The vocal fold vibration was observed with an ultrahigh-speed camera, which allowed rapid and fleeting aspects of the vocal fold movements to be seen with great detail. The most important observation was that the superior and inferior aspects of the vocal fold vibrate slightly out of phase. The inferior part of the vocal fold closes first and then is rapidly followed by the superior mass.

A very striking aspect of Farnsworth's[12] original observations of 2-mass vibration was that the closure of the inferior mass was clearly marked by a sharply demarcated ledge. Smith[22] replicated the Farnsworth experiment and published a larger series of clear pictures capturing the dynamic movements of the vocal folds. In these photographs the ledge of the inferior mass (he terms it the white mass) stands out starkly during the early stage of vocal fold closure. The superior mass (with the vocal ligament

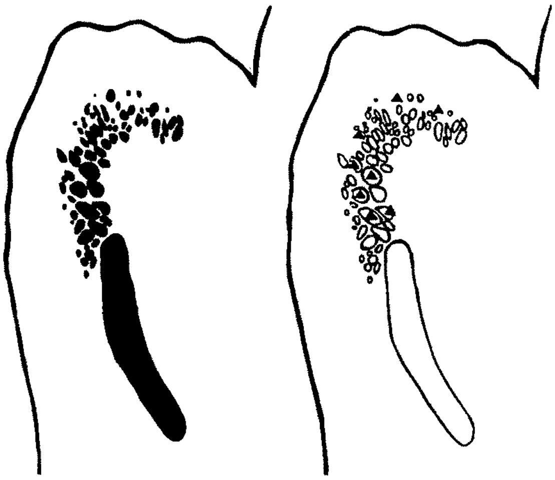

Fig 4. Comparison of muscle fascicle structure (left) to location of muscle spindles (right). In this specimen, 8 muscle spindles (triangles) are present, and all are located in superior vocalis subcompartment. In separate study,[21] it was found that 80% of all muscle spindles in thyroarytenoid muscle are localized to superficial part of superior vocalis subcompartment.

near its center) is seen above and lateral to the inferior ledge. The existence of this inferior ledge has been noted by other investigators viewing male vocal folds vibrating in the modal or chest register. This ledge must reflect some aspect of the deeper vocal anatomy. An early explanation of what the ledge might be was contained in the original paper by Hirano[23] that introduced the body-cover theory of vocal fold vibration. Hirano described the vocal fold as being composed of 2 layers with different vibratory characteristics; the body consists of the TA muscle and the conus elasticus, and the cover consists of the mucosa and lamina propria. In this view the primary vibrating element is the mucosal cover. The ledges (termed lips by Hirano) seen in human phonation are explained as deformations in the mucosal cover. In addition, the mucosal wave takes place primarily, if not exclusively, in the mucosa.

Certain experimental work in dogs can be useful as a model for exploring the deep biomechanical structure of the vocal fold. The canine TA muscle has a well-developed inferior vocalis SC and a distinct, albeit very small, superior vocalis SC.[16,24] Matsushita[25] viewed excised human and dog larynges from below while they were vibrating and noted that a ledge was seen in the subglottic mucosa. This ledge served as the initiation line of the mucosal wave, so it was named the mucosal upheaval. Yumoto et al[26] excised canine larynges and also viewed them from below while their vocal folds were artificially set into vibration. The area of the mucosal upheaval was marked so that it could be identified after the larynx was serially sectioned. Yumoto et al showed that the site of mucosal upheaval corresponds to the level at which the TA muscle came closest to the vocal fold surface. Although Yumoto et al do not comment on the underlying anatomy of the TA muscle, inspection of their micrographs suggests that the site of mucosal upheaval corresponds to the top of the inferior vocalis SC. Apparently, the passive biomechanical properties of the vocal fold in experimental studies allow vocal fold vibration in the superior vocalis SC, but not in the inferior vocalis SC. Although these experimental results are artificial and rely on the passive biomechanical properties of the vocal fold, they probably reflect aspects of how the vocal folds function in vivo. For example, Berke et al[27] have reported a phenomenon similar to mucosal upheaval in live dogs. In these experiments, the dogs were under general anesthesia, adduction of the vocal folds was done by electrically stimulating the recurrent nerves, and airflow was delivered by an intratracheal cannula. Although these conditions are greatly different from normal phonation in the awake animal, the vocal folds still showed a mucosal upheaval, even though the electrical stimulation must have recruited the entire TA muscle. Thus, as in the human, the dog TA muscle appears to have distinct superior and inferior vocalis SCs that vibrate differently under experimental conditions and thus may, in vivo, exhibit functionally distinct roles.

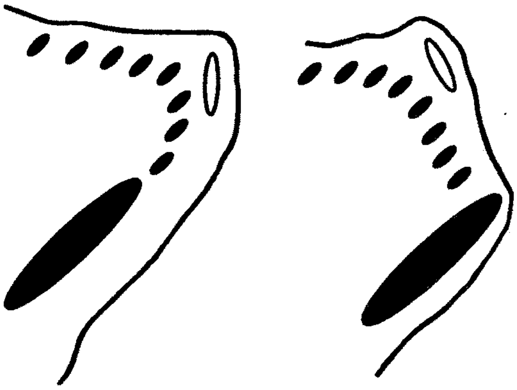

Fig 5. Schematic drawings showing basic muscle compartments of vocal fold at rest (left) and during closing phase of vocal fold vibration (right). In theory, during vocal fold closure, inferior vocalis subcompartment leads superior vocalis subcompartment and thereby forms sharp ledge that has been repeatedly seen during human phonation. White oval is vocal ligament.

The above-described observations of human and canine vocal fold vibration suggest a structure that corresponds to the human vocal fold ledge or canine mucosal upheaval. The only clear anatomic structures seen in the human vocal fold are the 2 SCs seen in this study. If the inferior SC leads the superior SC during vibration, the top of the inferior SC could constitute the ledge (Fig 5). As noted above, the possibility that the SCs are independently controlled is consistent with known facts regarding other muscle compartments. It is also consistent with the observations of Smith, who states that "the subglottic, white colored area increases in size when the intensity is increased and the pitch kept constant."[22(p168)] Therefore, the SCs not only are 2 separate masses, but they also are capable of changing their tension independently.

Smith's[22] data also have relevance for understanding the mucosal wave. In his observations, Smith makes note of the position of the vocal ligament throughout the vibratory cycle. He then correlates this with blood vessels that can be observed overlying the vocal ligament. He concludes that these blood vessels never move in relation to the vocal ligament. Therefore, the mucosal wave does not appear to involve movement of the cover in relation to the underlying body. Smith goes even farther and states that at no time does there appear to be a compression wave of the mucosa. Instead, the entire superior aspect of the vocal fold above the ledge rolls together until the entire vocal fold is closed. In essence, Smith argues that the undulation of the vocal fold surface is not a mucosal wave, but a progressive change in the shape of the whole superior lip of the vocal fold. This would include the superior SC, of course, and implies that this SC could directly control the shape and stiffness of the whole superior lip of the vocal fold.

Some observations of human vocal fold vibration can be interpreted as supporting the concept of an independently controlled superior vocalis SC. For example, it is generally accepted that during falsetto, the vocal folds are stretched thin, with vibration being almost entirely in the mucosal cover.[23] However, Pressman[7] noted that as the falsetto voice is raised to its highest pitches (loft registers), the posterior aspects of the vocal folds approximate. This dampens vibration and shortens the part of the vocal fold that can still vibrate. With increasing pitch, this dampening moves progressively in an anterior direction, until only a tiny anterior part of the vocal fold is left to vibrate.[7] As only the superior vocalis SC is near the vocal fold edge during falsetto, the progressive dampening that is seen must be due to recruitment of small parts of the superior vocalis SC. This suggests that not only is the superior vocalis SC controlled independently from the remainder of the TA muscle, but that smaller parts of the superior vocalis SC can be independently controlled. What these smaller parts consist of is unknown; however, the fascicular structure of the superior vocalis SC, and the distribution of muscle spindles throughout these small fascicles, may reflect this capability.

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UTILITY OF CONVENTIONAL RADIOGRAPHY IN THE DIAGNOSIS AND MANAGEMENT OF PEDIATRIC AIRWAY FOREIGN BODIES

ANDREW B. SILVA, MD
TACOMA, WASHINGTON

HARLAN R. MUNTZ, MD
ST LOUIS, MISSOURI

RANDALL CLARY, MD
ST LOUIS, MISSOURI

Pediatric airway foreign bodies are potentially life-threatening situations. The otolaryngologist is often consulted to aid in the diagnosis and management of these difficult cases. Although radiographic studies are often obtained, the decision for surgical intervention is usually based on a suspicious history and physical examination. Our hypothesis is that radiographic imaging should not alter the decision for surgical intervention. We retrospectively reviewed the cases of pediatric airway foreign bodies managed by the otolaryngology department at St Louis Children's Hospital between December 1990 and June 1996 with both radiographic imaging and operative intervention. Ninety-three cases of potential aspiration were identified, with a median patient age of 20 months. The most common presenting signs and symptoms were aspiration event (n = 82), wheezing (n = 76), decreased breath sounds (n = 47), cough (n = 39), respiratory distress (n = 17), fever (n = 16), pneumonia (n = 14), and stridor (n = 7). At the time of endoscopy, 73 patients were found to have an airway foreign body. The sensitivity and specificity of the imaging studies in identifying the presence of an airway foreign body in the 93 patients were 73% and 45%, respectively. Our decision for operative intervention was based on the history and physical examination, and was not changed in the presence of a negative radiographic study. The routine use of radiography should not alter the management of airway foreign bodies, providing that there is a well-equipped endoscopic team familiar with airway foreign bodies.

KEY WORDS — airway foreign body, aspiration, pediatrics, radiography, x-ray.

INTRODUCTION

Pediatric airway foreign bodies (FBs) are a potentially life-threatening situation. The otolaryngologist is often called upon to aid in the diagnosis and management of these difficult cases. The decision for operative intervention is based on a complete history and physical examination. Conventional x-ray imaging is usually obtained to aid in the diagnosis during the initial evaluation.

What should one do if the imaging studies do not support the diagnosis of an FB? Since the decision for operative intervention is based on a suspicious history and physical examination, the utility of obtaining radiologic imaging, with its additional cost, in these cases would be in question.

Our hypothesis is that radiographic imaging should not alter the decision for surgical intervention in patients with a suspicious history and appropriate findings on physical examination. In order to test this hypothesis, we retrospectively reviewed the cases of pediatric airway FB aspiration at St Louis Children's Hospital that had both radiologic imaging and operative intervention between December 1990 and June 1996.

MATERIALS AND METHODS

A retrospective review was conducted of patients treated at St Louis Children's Hospital for airway FBs between December 1990 and June 1996. Patients were included in this review if they were younger than 18 years of age, initially presented for an airway FB evaluation, and underwent radiographic imaging prior to operative intervention. Only children satisfying all 3 criteria were included. An airway FB was defined as an ingestion or aspiration of a foreign substance lodging between the hypopharynx and the distal bronchi.

Standard demographic factors, including patient age, sex, and prior diagnosis, were determined. The percentages of patients presenting with a cough, fever, wheezing, respiratory distress, decreased breath sounds, pneumonia, and stridor were calculated. The percentage of patients with a history of an aspiration event was determined along with the amount of time that elapsed between the aspiration event and surgery.

The number and type of different x-ray studies were recorded for each patient. The charts and official reports of each x-ray study were reviewed to

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From the Department of Otolaryngology–Head and Neck Surgery, Washington University School of Medicine, St Louis, Missouri.

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CORRESPONDENCE — Andrew B. Silva, MD, Dept of Otolaryngology, Madigan Army Medical Center, Fort Lewis, Tacoma, WA 98431.

TABLE 1. LOCATIONS OF 74 EXTRACTED FOREIGN BODIES IN 73 PATIENTS

	No. of Foreign Bodies	
Airway Location	*No.*	*%*
Larynx	5	7
Trachea	10	14
Right main stem	22	30
Left main stem	17	23
Right bronchus	16	21
Left bronchus	4	5
Total	74	100

determine the type of radiographic abnormality, its location within the airway, and if it was suggestive of an airway FB. These factors were compared to the intraoperative findings at the time of surgical intervention (direct laryngoscopy and bronchoscopy).

The time interval between the aspiration event and the x-ray study was determined in each case. The study population was grouped by different time intervals depending on the amount of time that had elapsed. The sensitivity and specificity of the x-ray studies in identifying the presence of an airway FB were determined for the entire study group and for each time interval.

RESULTS

Ninety-five patients were evaluated for an airway FB at St Louis Children's Hospital between the months of December 1990 and June 1996. Ninety-three patients were identified as having radiographic imaging prior to operative intervention. (Two patients were excluded from the study because they did not undergo radiographic imaging prior to surgical intervention.) Their ages ranged from 6 months to 168 months with a median age of 20 months. The study group contained 64 boys and 29 girls with a male-to-female ratio of 2.2:1.

Prior to admission, the patients (n = 93) received the following diagnoses or misdiagnoses: 17 with reactive airway disease (RAD), 6 with pneumonia, 3 with croup, and 67 with an airway FB. At the time of endoscopy, 88% (15/17) of the patients with RAD, 83% (5/6) of the patients with pneumonia, 33% (1/3) of the patients with croup, and 78% (52/67) of the patients with an airway FB had an airway FB removed. A total of 73 of 93 patients, 78% of our study group, had an airway FB.

The following signs and symptoms were noted during the initial evaluation: wheezing (76/93, 82%), decreased breath sounds with auscultation (47/93, 51%), cough (39/93, 42%), respiratory distress (17/93, 18%), fever (16/93, 17%), pneumonia (14/93, 15%), and stridor (7/93, 8%). A history of an aspiration event was elicited in 82 patients (88%), and 65 (80%) of these patients had an FB at the time of endoscopy. Eleven patients (12%) presented without an aspiration event, and 8 (73%) patients had an FB at the time of endoscopy. In 9 (82%) of these 11 patients, the preoperative x-ray studies were suggestive of an FB and prompted referral for endoscopy. In the remaining 2 patients (18%), the x-ray studies were not suggestive of an FB, but the patients were referred on the basis of their respiratory disease.

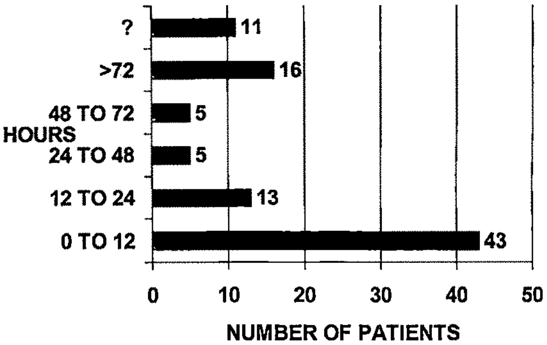
TABLE 2. COMPOSITION OF 74 FOREIGN BODIES EXTRACTED IN 73 PATIENTS

	No.	*%*
Plastic	13	17
Metal	7	9
Vegetable (nuts)	52	70
Candy	2	3
Total	74	100

A total of 146 radiologic studies were used to evaluate 93 patients: 88 chest radiographs, 15 inspiratory and expiratory chest radiographs, 7 lateral chest decubitus radiographs, 22 fluoroscopic studies of the chest, and 14 cervical airway films. These studies were suggestive of an airway FB in 64 patients (69%). Of these 64 patients with a radiologic study suggestive of an FB, 53 (83%) were found to have an FB at the time of endoscopy and 11 (17%) were not. A total of 71 radiographic abnormalities were noted in these 64 patients: 9 radiopaque FBs, 21 studies with pulmonary consolidation, 34 studies with air trapping, and 7 studies with pulmonary atelectasis.

Seventy-four foreign bodies were retrieved from 73 patients in our study group. Only 1 patient was found to have 2 foreign bodies at the time of endoscopy. The location and type of the 74 FBs in our 73 patients are listed in Tables 1 and 2, respectively. The time intervals between the aspiration event and the operative intervention are shown in the Figure.

The sensitivity and specificity of conventional ra-



Elapsed time from aspiration event to surgery in patients with and without history of aspiration event (n = 93).

TABLE 3. COMPARISON OF RADIOGRAPHY TO ENDOSCOPY ONLY IN ALL PATIENTS, WITH AND WITHOUT HISTORY OF ASPIRATION EVENT (N = 93)

	+FB	*–FB*
+ Radiograph	53	11
– Radiograph	20	9

73% Sensitivity, 45% specificity.
FB — foreign body.

diographs in the diagnosis of an airway FB were 73% and 45%, respectively, in our study group of 93 patients (Table 3). In order to determine if there was a time-dependent advantage to ordering radiographs in the population of patients with a history of an aspiration event (n = 82), the sensitivity and specificity were calculated for radiographs obtained <24 hours and >24 hours after the aspiration event. The sensitivity and specificity were 63% and 47% for the radiographs taken within the first 24 hours (Table 4) and were 83% and 50% for radiographs taken after the first 24 hours (Table 5) following the aspiration event. The sensitivity and specificity for the patient group with no prior history of an aspiration event (n = 11) were 88% and 33%, respectively.

DISCUSSION

Pediatric airway FBs are potentially life-threatening situations that occur most commonly in infants and toddlers, with a male-to-female ratio of 2:1. The classic presentation of an aspiration event — paroxysmal coughing, wheezing, and decreased breath sounds on the affected side — is not always seen. The diagnosis of a pediatric airway FB can be difficult, since they most commonly occur in infants and toddlers who are unable to readily communicate the circumstances surrounding the aspiration event, which may or may not have been witnessed. The location and composition of the extracted FBs in our series are consistent with those reported in the literature.[1-3]

Conventional radiographs are often used in the diagnosis of airway FBs. Esclamado and Richardson[4] reported that soft tissue posteroanterior and lateral cervical airway films are superior to chest x-ray studies in the diagnosis of a laryngotracheal FB. In their series of 19 patients with a laryngotracheal FB, 19 patients had chest x-ray studies and 13 patients had airway films. Only 8 of the 19 patients with chest x-ray studies had abnormal findings, while 12 of the 13 patients with cervical airway films had abnormal findings. Halvorson et al[5] reported that 5 of 7 patients with a subglottic FB in their series had diagnostic cervical airway films. We report 5 patients with a laryngeal FB: 3 patients had cervical airway films, and 2 films were diagnostic.

Patients with a laryngeal FB are at a high risk for complete airway obstruction, and if this diagnosis is suspected, it might be prudent not to delay endoscopy to obtain films. Kent and Watson[6] reported on 4 cases of laryngeal FBs. Two patients had diagnostic cervical airway films before endoscopic removal, and 2 patients acutely obstructed in the emergency room, requiring emergent tracheotomy, with 1 death.

Tracheobronchial FBs are commonly imaged with chest x-ray studies. The sensitivity and specificity of conventional x-ray studies in the diagnosis of an airway FB were 73% and 45%, respectively, in our study. Svedstrom et al[7] reported sensitivity and specificity of 68% and 67%, respectively, in their series of 83 patients. Thirty-four patients had an FB diagnosed at endoscopy: 50% had air trapping, 18% had signs of infection, 12% had atelectasis, and 24% had no abnormality on their chest x-ray studies. Svedstrom et al concluded that chest x-ray studies are neither sensitive nor specific enough to diagnose an airway FB. Our data support this statement. If the decision to proceed with endoscopy had been based on abnormal radiographic findings, 20 patients in our series with an airway FB would not have gone for bronchoscopy.

A high index of suspicion for an airway FB must be maintained during the evaluation of a patient with a history of an aspiration event or suspicious respiratory symptoms. Prior to our evaluation, 26 patients had been treated for the following conditions: RAD (17), pneumonia (6), and croup (3). It is interesting to note that 15 of the 17 patients with a prior diagnosis of RAD, 5 of the 6 patients with pneumonia, and 1 of the 3 patients with croup had an FB identified at endoscopy. The factors in these patients' histories that prompted endoscopic evaluation were symptoms

TABLE 4. COMPARISON OF RADIOGRAPHY TO ENDOSCOPY ONLY IN PATIENTS WITH HISTORY OF ASPIRATION EVENT (N = 82) AND RADIOGRAPHS OBTAINED <24 HOURS FROM THEIR ASPIRATION EVENT (56/82)

	+FB	*–FB*
+ Radiograph	26	8
– Radiograph	15	7

63% Sensitivity, 47% specificity.

TABLE 5. COMPARISON OF RADIOGRAPHY TO ENDOSCOPY ONLY IN PATIENTS WITH HISTORY OF ASPIRATION EVENT (N = 82) AND RADIOGRAPHS OBTAINED >24 HOURS FROM THEIR ASPIRATION EVENT (26/82)

	+FB	*–FB*
+ Radiograph	20	1
– Radiograph	4	1

83% Sensitivity, 50% specificity.

refractory to medical treatment, a new onset of symptoms in a healthy child, or an acute exacerbation of an underlying disease process.

If a high index of suspicion is not maintained, then a delay in diagnosis can occur. A delay in diagnosis of >24 hours in 40% to 72% of cases has been noted in several studies.[8-10] There was an overall delay in diagnosis of >24 hours of 40% in our study, and even a delay in diagnosis of 32% in the group of patients with a history of an aspiration event. Increased morbidity, mortality, and inappropriate treatment have been reported in patients with a delay in diagnosis.[8,11] In our series, 15 of the 17 patients were inappropriately treated for RAD prior to recognition of an airway FB, causing a delay in diagnosis of >24 hours in all the cases. Eleven of the 14 patients who presented with pneumonia also had a delay in diagnosis of >24 hours, increasing their morbidity.

Prompt surgical intervention should lower the mortality and morbidity associated with an FB aspiration. Patients presenting shortly after an aspiration event run the risk of a bronchial FB dislodging, causing acute obstruction of the laryngotracheal airway. In 1 of our cases, the decision was made to observe and monitor a patient overnight. In the morning the patient developed acute respiratory distress and was emergently taken to the operating room. The FB had dislodged from its distal impaction site to occupy a more proximal position in the trachea, causing near-total obstruction. Patients with acute symptoms or a new onset of symptoms associated with an airway FB should urgently undergo endoscopy as soon as preparations for the operating room can be made, the equipment is tested, and qualified personnel are available. Patients who present after the acute event will have a longer history of symptoms. In these cases, endoscopy can be carried out electively as soon as arrangements can be made.

The decision for surgical intervention is based on a suspicious history and physical examination. A history of an aspiration event can be elicited in 73% to 85% of all patients with an airway FB.[12] A history of an aspiration event was elicited in 88% of our study group. In the presence of an aspiration event and/or a suspicious history and physical examination, the decision for surgical intervention is generally made before the x-ray studies are ordered. Radiography in this population would not be cost-effective, since one is obligated to endoscopically examine the airway to rule out an FB. The sensitivity and specificity of radiographic imaging are not sufficient to allow for the diagnosis of an FB. Even if the study is negative, the presence of an airway FB cannot be excluded, obligating the endoscopy and airway examination.

The sensitivity and specificity of radiography performed within the first 24 hours after the aspiration event were compared with those of radiography performed after the first 24 hours to determine if a longer time frame would improve their yield. No significant difference was noted between the two groups (Tables 4 and 5). This implies that there is no advantage to selectively obtaining radiographs in patients who present after the first 24 hours from an aspiration event.

Eleven patients in our series did not have a history of an aspiration event. In these 11 cases, radiographic abnormalities prompted the referral to our department for evaluation. At endoscopy, 8 were found to have an airway FB. The use of radiography in patients without a history of an aspiration event was very helpful. Identification of a segmental radiologic abnormality such as obstructive emphysema, air trapping, atelectasis, consolidation, or a mediastinal shift will raise the suspicion for an airway FB and prompt the appropriate referral.

CONCLUSION

The diagnosis of an airway FB is based on a suspicious history and physical examination, especially a history of an aspiration event. This would obligate the endoscopist to examine the airway regardless of the radiographic results. In these cases, the routine use of radiography is probably neither efficient nor cost-effective. Exceptions to this general rule may include patients who need their pulmonary status evaluated prior to transportation to a tertiary care center, or patients with a complicated presentation in whom a baseline against which improvement can be noted would be of value.

In cases without a suspicious history and physical examination, radiography is often very helpful. Identification of a segmental radiographic abnormality will often prompt the referral of a patient to a medical center familiar with the diagnosis and treatment of airway FBs. Radiography can also help determine the number, type, and location of radiopaque FBs, especially in cases in which the aspiration was not witnessed.

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TRAUMATOLOGY OF THE HEAD AND NECK

The Berlin Course on Traumatology of the Head and Neck (with osteosynthesis exercises and live operation) will be held November 5-7, 1998, in Berlin, Germany. The special topic will be acceleration-deceleration trauma (whiplash). For information, contact Prof Arne Ernst, Dept of Otolaryngology at UKB, Rapsweg 55, D-12683 Berlin, Germany; telephone 030 5681 2801; fax 030 5681 2903; e-mail ernst.orl.ukberlin@t-online.de.

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POSTERIOR GLOTTIC STENOSIS: A CANINE MODEL

MARK S. COUREY, MD
GRADY LEE BRYANT, JR, MD ROBERT H. OSSOFF, DMD, MD
NASHVILLE, TENNESSEE

Posterior glottic stenosis is a disabling disease in which the vocal folds are fixed near the midline. This allows adequate vocal fold adduction for voicing, but does not permit useful abduction for ventilation. The most common cause is prolonged endotracheal intubation for mechanical ventilation, and the incidence is estimated at 4% for intubations between 5 and 10 days. Currently, our understanding and treatment modalities are based on retrospective reviews of small, nonrandomized clinical experiences. The purpose of this project was to develop an animal model that would improve our understanding of histologic changes and allow future prospective randomized trials for therapeutic intervention. Twelve dogs, 15 to 25 kg, were randomly divided into three groups. Animals in group 1 had a superficial injury produced in the tissue over the right cricoarytenoid joint; animals in group 2 had a deep soft tissue injury produced; and animals in group 3 underwent joint opening. The animals were allowed to recover for 2 months. Morphometric analysis of the harvested larynges demonstrated clinically significant limitation in motion in the animals with deep soft tissue injury and in animals with joint disruption. Histologic analysis revealed various degrees of injury, from loss of subepithelial soft tissue to cartilaginous resorption and fusion of the arytenoid to the cricoid. These findings were directly related to the depth of the initial injury. It is possible to produce posterior glottic stenosis in the canine species. This will serve as a reliable animal model for future study.

KEY WORDS — arytenoid fixation, canine, larynx, posterior glottic stenosis, vocal fold paralysis.

INTRODUCTION

Posterior glottic stenosis (PGS) is a disabling disease. The vocal folds are fixed near the midline. This allows adequate voicing, but does not permit abduction for ventilation. Trauma from prolonged intubation for mechanical ventilation is the most common cause.[1,2] The incidence of PGS is approximately 4% in adults intubated between 5 and 10 days and 14% in adults intubated between 10 and 24 days.[1] As our ability to support patients with mechanical ventilation improves, we, as otolaryngologists, can expect to see an increasing incidence of PGS. Currently, there are no universally accepted standards for the management of patients requiring prolonged intubation. Specifically, attempts to define length of safe intubation time prior to the induction of PGS have been unsuccessful. Other factors, such as bacterial overgrowth, gastroesophageal reflux, patient and tube management including size of the endotracheal tube and the use of paralytic agents, and patient genetics with regard to wound healing, all influence the likelihood of the development of PGS.[3]

Attempts to better understand PGS through examination of multiple large patient series has led to the knowledge that PGS can occur secondary to either adhesions between the arytenoids or scarring within the plane of the cricoid ring and cricoarytenoid joint.[1,4] The latter process is significantly more common and may be confused with bilateral vocal fold paralysis. Electromyography (EMG), in patients with known nerve injury, frequently reveals normal voluntary motor unit potentials. Therefore, differentiation of PGS from bilateral paralysis often requires direct endoscopy with palpation. Palpation, however, only discloses overall joint mobility. Information about joint space disruption and the location of mobility limitation can only be gained through histologic examination of the joint. This is not possible in living patients. The exact site of the injury that limits arytenoid motion may have an important influence on the success of surgical procedures for the treatment of PGS.

Posterior glottic stenosis needs to be differentiated from bilateral vocal fold paralysis because restoration of joint mobility in PGS will permit return of dynamic function, thus allowing both voicing and ventilation.[5] The treatment of bilateral paralysis, however, must rely on either nerve replacement,[6-9] regeneration from end-to-end anastomosis,[10,11] or electrical pacing.[12-14] None of these modalities are

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From the Department of Otolaryngology, Vanderbilt University Medical Center, Nashville, Tennessee. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Vanderbilt University Medical Center.

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CORRESPONDENCE — Mark S. Courey, MD, Dept of Otolaryngology, Vanderbilt Voice Center, The Village at Vanderbilt, 1500 21st Ave S, Suite 2700, Nashville, TN 37212.

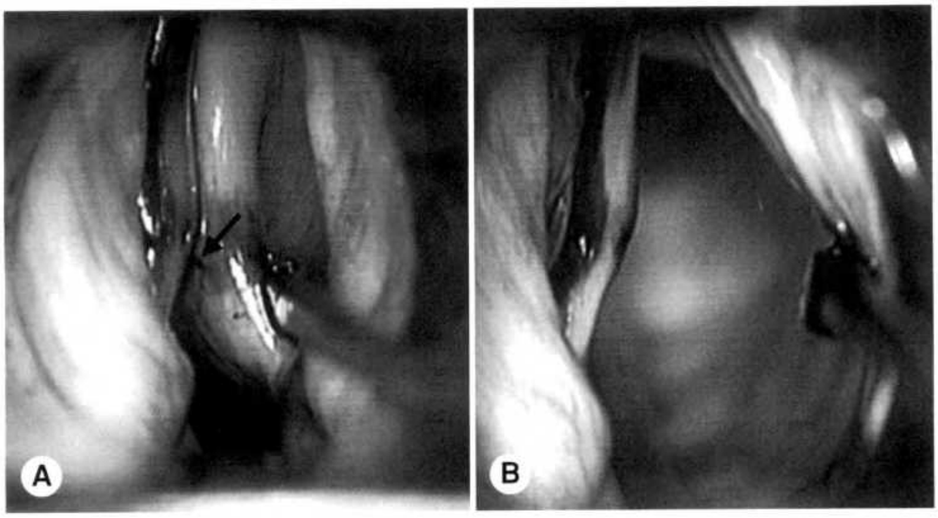

Fig 1. Endoscopic view of full passive abduction of vocal folds (original ×6.8). A) Arrow — India ink tattoo over vocal process. B) Same animal.

currently available. Therefore, ablative procedures to statically widen the posterior glottis may be employed. These procedures, such as arytenoidectomy, are referred to as the great compromise.[15] They improve the size of the airway; however, they decrease glottic closure and inhibit voicing. In addition, disruption of glottic closure may lead to aspiration. These static procedures may, at times, be used in the treatment of PGS. For the previously stated reasons, this is less than ideal, and attempts to restore dynamic function prior to proceeding with ablative procedures that permanently alter voice are indicated.

Procedures for the treatment of PGS are directed at removal or release of the scar tissue that limits arytenoid movement. These include dilation, incision and dilation, posterior microtrapdoor flap for excision of submucosal scar tissue, and open procedures with grafting of skin and/or cartilage to the posterior glottis.[16] All of these procedures have limited success, and in addition, procedures involving grafting often result in permanent widening of the posterior glottis, which inhibits voice production.[17] Because of limited success with treatment, the creation of an animal model would allow better study of the disease process and prospective therapeutic management trials. This would lead to improved capabilities for restoration of vocal fold mobility. The purpose of this research was therefore to create an animal model for PGS for future study.

METHODS AND MATERIALS

Experimental Procedure. Secondary to similarities with humans in laryngeal size and cricoarytenoid joint configuration, the dog was chosen as the animal model for study. Twelve adult mongrel dogs (11 male and 1 female) were randomly divided into three groups. Dogs ranged in weight from 15 to 25 kg. All dogs underwent direct suspension microlaryngoscopy with thiopental sodium anesthesia. The larynx was exposed through an Ossoff-Karlan modified Dedo Laryngoscope (Pilling-Weck, Research Triangle Park, NC) and suspended with a table mount suspension device. The surgical field was viewed through a surgical microscope (OPMI 1, Zeiss, Germany). The cricoid and arytenoid cartilages were palpated with a straight blunt probe. India ink was placed submucosally over the tip of the vocal process of the arytenoid, over the midpoint of the body of the arytenoid at the level of the vocal fold, and in the midline of the posterior commissure. Cricoarytenoid joint mobility was assessed by manipulating the tip of the vocal process of the arytenoid and observing vocal fold movement. Photodocumentation of resting position and maximum abduction and adduction was obtained with a video camera (MKC-301A, Ikegami, Tokyo, Japan) and input into a Macintosh computer (Macintosh 840 AV) with a built-in video card (Apple Computer Co, Cupertino, Calif; Fig 1).

At the same operative procedure, a 1-cm^2 area over the right cricoarytenoid joint space was identified. The area was defined by cutting a cottonoid patty into a 1-cm square and centering it over the joint space. Carbon dioxide (CO_2) laser energy (Sharplan model 1069 CO_2 laser, Sharplan, Allendale, NJ) was delivered through a Reliant Unimax 2000 Microspot Micromanipulator (Reliant Technologies, Foster City, Calif) attached to the operating microscope with a 400-mm focal length lens. The edges of the 1-cm^2 area to be treated were marked with the laser on 6.0-W single pulse. The cottonoid was removed and the laser was used to ablate tissue over the joint space to a specified level.

Group 1: Superficial Injury. The laser was used in a slightly defocused mode at 6 W superpulse to ablate tissue over the joint space in a cross-hatching manner. Carbonaceous debris was cleaned from the

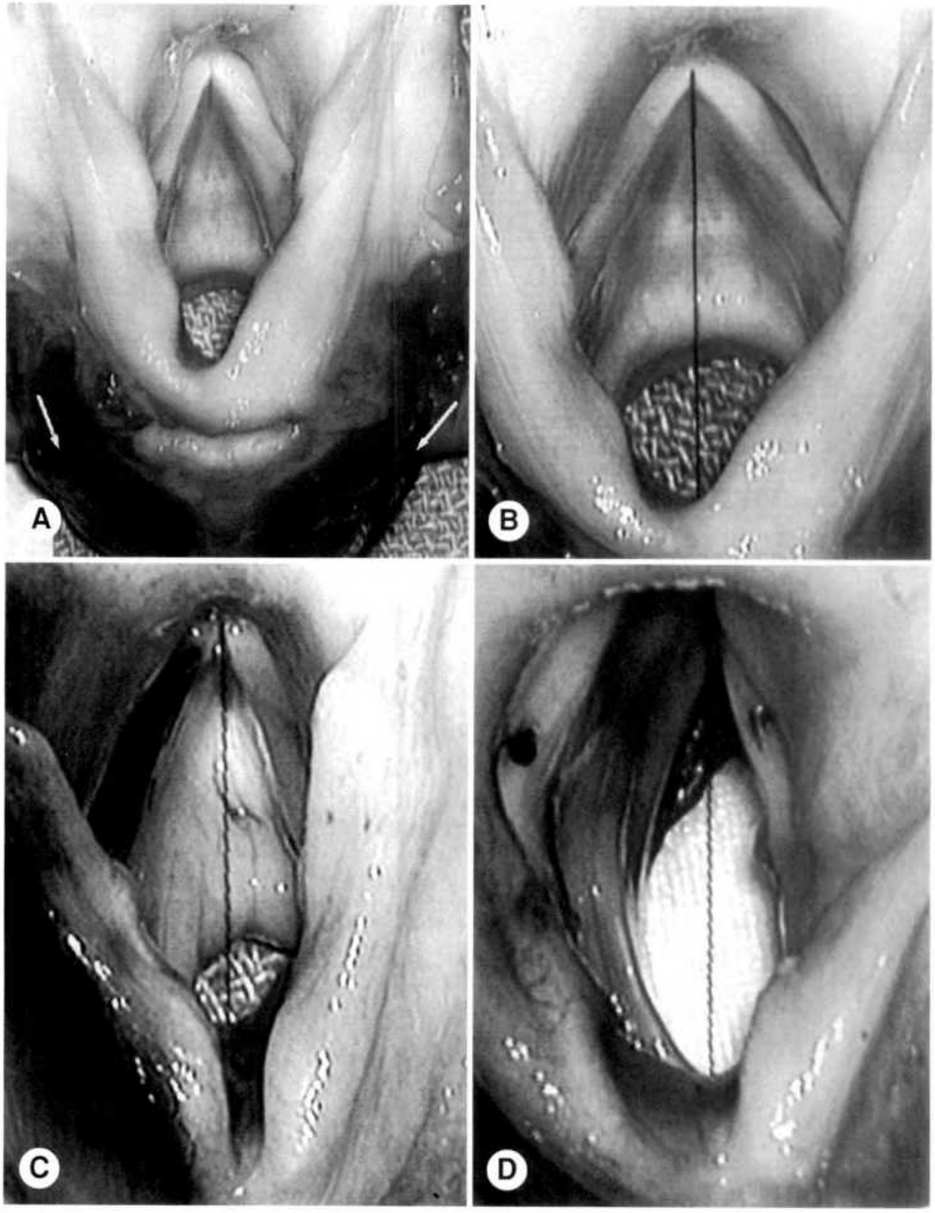

Fig 2. Harvested larynges. A) At rest. Arrows — 2-0 silk suture in vocal process. B) Group 1 animal, full abduction. Centerline is used to compare right and left glottal areas. C) Group 2 animal, full abduction. D) Group 3 animal, full abduction.

field with a saline-moistened cottonoid, and then a second pass with the laser at the same power settings was performed.

Group 2: Deep Soft Tissue Injury. The laser was used at the same power settings to ablate tissue to the level of the cricoid and arytenoid mucoperichondrium. Multiple passes were made with the laser until cricoid cartilage was exposed at the lower edge of the wound. Once cartilage was identified, through visualization of a characteristic change of incendiary characteristics, the procedure was terminated.

Group 3: Joint Injury. In this last group of animals, after exposure of the joint capsule, the capsule itself was ablated. The upper border of the cricoid and the lower border of the arytenoid were exposed, and perichondrium was ablated.

Recovery and Evaluation. All animals were housed for 2 months in an approved animal care facility. They were resuspended under thiopental anesthesia, the operative site was exposed, and the cricoarytenoid joints were palpated with a blunt probe. Mobility, both adduction and abduction, on the operative (experimental) side was compared to that on the nonoperative side. In this manner each animal served as its own control. Characteristics of the overlying mucosa were assessed. Photographs were taken.

A lethal dose of sodium pentobarbital (300 mg/kg intravenously) was administered and the larynx harvested. Mucosa over the muscular process of the arytenoids was removed and a 2-0 silk suture placed through each muscular process. The larynx was then

TABLE 1. GROUP 1 (SUPERFICIAL INJURY)

	Area in Abduction (mm²)			*Distance From Midline to VP (mm)*		*Area at Rest (mm²)*		*Distance From Midline to VP (mm)*	
Dog No.	*Left*	*Right*	*% Change*	*Left*	*Right*	*Left*	*Right*	*Left*	*Right*
290	67.06	79.37		5.30	5.78	41.19	38.75	3.51	3.89
331	89.60	31.52	65	6.83	3.71	17.49	28.93	1.52	3.24
335	69.24	70.51		6.01	5.87	54.74	60.04	3.93	3.65
349	70.70	53.44	25	5.65	4.90	46.10	29.05	2.30	2.17

VP — vocal process.

mounted on a wooden board with 2-0 silk sutures placed through the epiglottis anteriorly and the superior thyroid ala posteriorly. The superior surfaces of the vocal folds were exposed and visualized through the operating microscope with the attached Ikegami video camera. A metric ruler was photographed at the laryngeal level with each specimen (Fig 2A). By manipulating the muscular process sutures, the arytenoids were placed into either physiologic abduction or adduction by pulling in the direction of the posterior cricoarytenoid muscle fibers or in the direction of the lateral cricoarytenoid–thyroarytenoid complex muscle fibers, respectively. With equal tension bilaterally, maximal abduction and adduction could be achieved. Photographs were taken of the adducted, neutral, and abducted positions.

Morphometric Analysis. Neutral and abducted photographs of the glottis were analyzed with the NIH Image program (public domain software developed at the National Institutes of Health). With the Draw portion of the program, a "centerline" was drawn from the anterior commissure to the midline of the posterior commissure. The previously placed posterior midline tattoo served as a guide for centerline placement. The distance from the centerline to the most lateral portion of the vocal fold, near the tip of the vocal process, was measured at a 90° angle to the centerline. This was done on both the experimental (right) side and the control (left) side with the larynx in the neutral position and in full abduction.

Glottal area was also assessed with the NIH Image program. The outline of the glottal area from either side of the centerline was traced with the computer mouse on the screen and then computed in square millimeters with the Area portion of the program. With tension on the muscular process sutures producing full abduction, area was compared from the experimental (right) side to the control (left) side. A percentage change was calculated with the following formula: Change% = 100 − (experimental area/control area)100. The area from the neutral position was also compared with the ipsilateral area during maximal abduction.

Histologic Analysis. After the morphometric analysis, the larynx was split sagittally in the midline. The entire arytenoid with the underlying cricoid ring was excised. The specimen was further divided in a rostral-to-caudal plane through the middle of the cricoarytenoid joint, placed into cassettes, and fixed in 10% formalin. The tissue was embedded in paraffin, and 8-μm-thick sections were cut from the surface of each block through the joint space. Hematoxylin and eosin and Masson's trichrome staining were used to assess histologic changes.

RESULTS

At the initial operative procedure, prior to injury, all dogs had normal passive cricoarytenoid joint mobility bilaterally as assessed through direct microlaryngoscopy. When the mobilities of the right and left cricoarytenoid joints were compared within each animal, they were essentially equal in magnitude. Due to variations in laryngeal size and configuration among the animals, it was not possible to

TABLE 2. GROUP 2 (DEEP SOFT TISSUE INJURY)

	Area in Abduction (mm²)			*Distance From Midline to VP (mm)*		*Area at Rest (mm²)*		*Distance From Midline to VP (mm)*	
Dog No.	*Left*	*Right*	*% Change*	*Left*	*Right*	*Left*	*Right*	*Left*	*Right*
321	53.63	40.69	25	4.59	3.36	24.85	25.06	2.17	2.74
339	97.42	49.36	49	6.35	3.17	36.49	29.89	2.80	3.12
370	84.82	25.49	71	7.54	2.38	23.19	24.08	1.98	1.75
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*Information not available.

TABLE 3. GROUP 3 (JOINT INJURY)

	Area in Abduction (mm^2)			*Distance From Midline to VP (mm)*		*Area at Rest (mm^2)*		*Distance From Midline to VP (mm)*	
Dog No.	*Left*	*Right*	*% Change*	*Left*	*Right*	*Left*	*Right*	*Left*	*Right*
286	60.55	14.40	76	6.61	1.26	26.94	7.54	2.99	0.87
288	58.15	7.62	87	4.81	1.18	4.33	1.55	1.07	0.45
320	82.96	28.26	66	6.18	2.12	30.52	12.62	2.39	0.62
372	63.30	57.45	8	4.53	4.70	12.61	41.98	1.17	3.82

derive an absolute magnitude of abduction for the group as a whole.

One dog in the deep soft tissue injury group (group 2) developed airway distress on postoperative day 28. The dog was euthanized to prevent suffering. Examination of the larynx after harvest revealed a granuloma arising from the middle of the operative field. Histologic examination demonstrated significant resorption of the cricoid ring with marked inflammation.

Morphometric Findings. Eleven animals were available for analysis 2 months after the original injury was produced. Four were in group 3, 3 in group 2, and 4 in group 1. Maximal abduction was reduced in all group 2 and 3 animals to a variable extent. With 1 exception, the animals in group 1 had less than a 25% change in glottal area from the control (left) side to the experimental (right) side during maximal abduction (Fig 2B). The arytenoids on the experimental (right) side retained mobility, with the distance from the midline to the vocal process changing by greater than 2.22 mm from the neutral position to the position of maximal abduction. On the control (left) side, the distance from the midline to the vocal process changed by greater than 1.79 mm (Table 1).

Only 3 group 2 animals (deep soft tissue injury) were available for morphometric analysis. Change in glottal area from the control (left) side to the experimental (right) side ranged from 25% to 71% during maximal abduction (Fig 2C). All arytenoids on the experimental (right) side showed a significant reduction in motion, with the distance from the midline to the vocal process changing by less than 0.63 mm from the neutral position to the position of maximal abduction. On the control (left) side, the distance from the midline to the vocal process changed by greater than 2.42 mm (Table 2).

Three of the 4 group 3 animals (cartilage injury) showed a 66% to 87% reduction in glottal area at full abduction when the control (left) side was compared with the experimental (right) side (Fig 2D). The 1 outlier showed only an 8% reduction. However, the arytenoid on the experimental side was completely immobile, and the distance from the midline to the vocal process varied by 0.88 mm from the neutral position to full abduction, while it changed 3.46 mm on the control (left) side (Table 3).

Histologic Findings. Histologic examination of the control joints revealed a synovial joint space lined with a low cuboidal epithelium. On the luminal side of the joint, a fibrous connective tissue band was identified running between the arytenoid and cricoid cartilages. Overlying this band were glandular elements and respiratory epithelium. A transition zone from squamous epithelium to respiratory epithelium was seen overlying the midportion of the arytenoid body (Fig 3A). Muscular attachments were well visualized on the muscular process of the arytenoid.

In all group 1, 2, and 3 animals, re-epithelialization of the luminal surface of the larynx had occurred by 2 months. This was a respiratory epithelium with a transition to squamous epithelium over the body of the arytenoid. There was a complete loss of submucosal glandular elements in the experimental tissue. In group 1 and 2 animals, the joint space was maintained. Group 2 animals had a thicker fibrous band overlying the joint space when compared to group 1 animals. In addition, all group 2 animals showed some remodeling of the cricoid ring where it had been injured interiorly to identify the plane of dissection. Finally, group 3 animals showed a complete loss of the joint space with a dense scar band connecting the cricoid and arytenoid cartilages, significant remodeling of both the arytenoid and cricoid cartilages, and resorption of a large portion of the arytenoid cartilage. In 2 of the 4 animals a cartilaginous bridge could be seen nearly fusing the cartilages (Fig 3B,C).

Animal 331, a group 1 (superficial injury) animal, demonstrated a 65% reduction in glottal area on the experimental (right) side when compared to the control (left) side. Histologic analysis revealed significant resorption of the arytenoid cartilage, with thick fibrous tissue and neovascularization over the joint space. This was more characteristic of a group 2 injury.

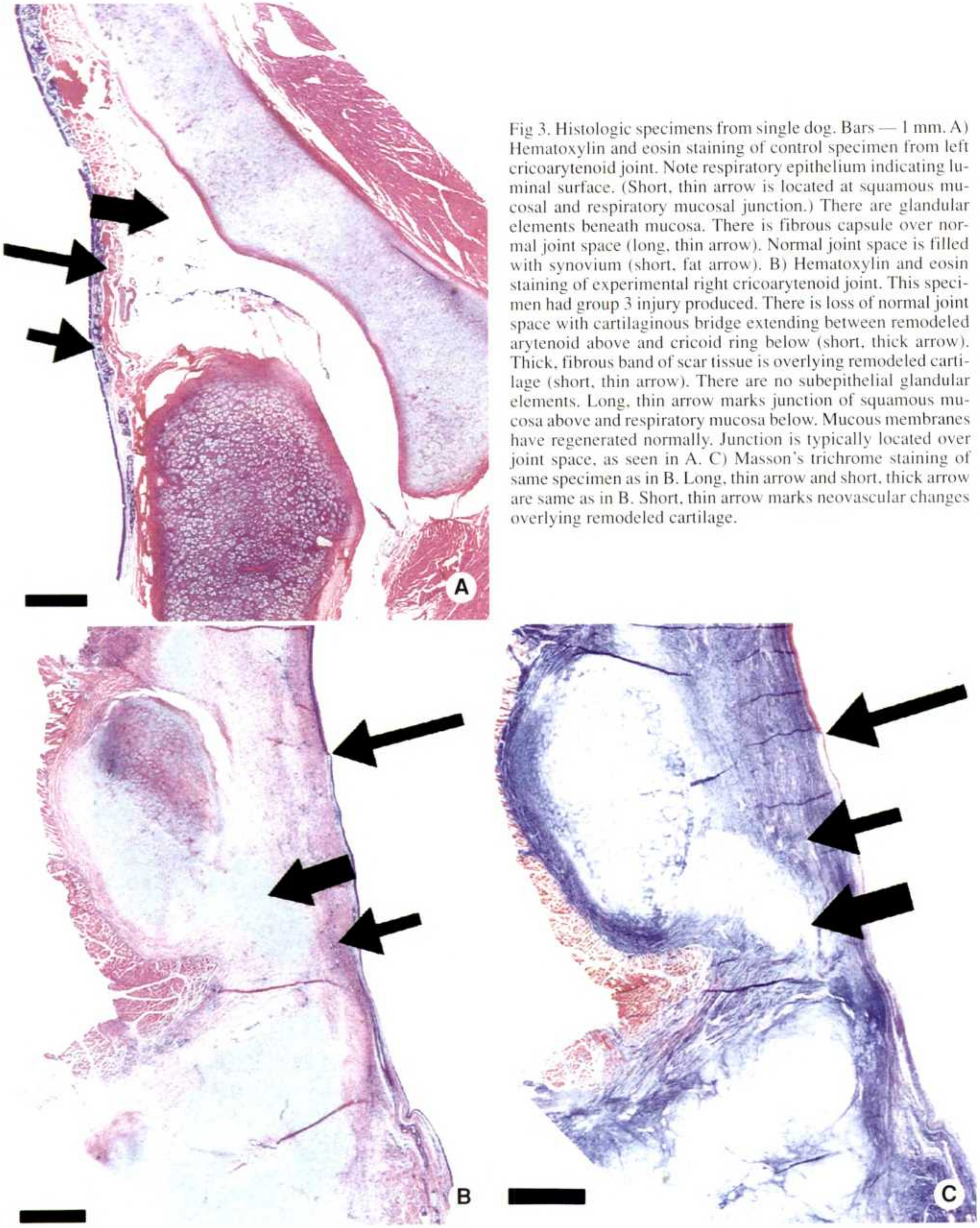

Fig 3. Histologic specimens from single dog. Bars — 1 mm. A) Hematoxylin and eosin staining of control specimen from left cricoarytenoid joint. Note respiratory epithelium indicating luminal surface. (Short, thin arrow is located at squamous mucosal and respiratory mucosal junction.) There are glandular elements beneath mucosa. There is fibrous capsule over normal joint space (long, thin arrow). Normal joint space is filled with synovium (short, fat arrow). B) Hematoxylin and eosin staining of experimental right cricoarytenoid joint. This specimen had group 3 injury produced. There is loss of normal joint space with cartilaginous bridge extending between remodeled arytenoid above and cricoid ring below (short, thick arrow). Thick, fibrous band of scar tissue is overlying remodeled cartilage (short, thin arrow). There are no subepithelial glandular elements. Long, thin arrow marks junction of squamous mucosa above and respiratory mucosa below. Mucous membranes have regenerated normally. Junction is typically located over joint space, as seen in A. C) Masson's trichrome staining of same specimen as in B. Long, thin arrow and short, thick arrow are same as in B. Short, thin arrow marks neovascular changes overlying remodeled cartilage.

DISCUSSION

Posterior glottic stenosis is a poorly understood process most commonly resulting from prolonged endotracheal intubation for mechanical ventilation. The purpose of this exercise was to develop an animal model to further the understanding of the histologic changes that occur in PGS and for future prospective study of diagnostic techniques and therapeutic interventions. Due to similarities with the human larynx in size and configuration, the canine larynx provided an excellent model for this work.

Injury over the cricoarytenoid joint capsule was produced at three different known depths to simulate erosion from endotracheal tube placement. Presumably, relatively short periods of intubation would result in superficial injury with mucosal and subepithelial tissue loss, while longer periods of intubation, with or without other factors such as extraesophageal reflux, would lead to erosion of deeper tissues with exposure of the cricoarytenoid joint capsule and/or actual erosion into the joint with perichondrial disruption and cartilaginous injury.

All experimental animals demonstrated histologic changes consistent with previous injury. Specifically, loss of subepithelial glandular tissue with an increase in fibrous scar tissue and neovascularization were seen in even the group with the most superficial injuries. The laryngeal mucosa, however, regenerated successfully without significant changes when compared to the normal, uninvolved side.

The degree to which cricoarytenoid joint mobility was limited was directly dependent on the depth of injury. While superficial injury produced histologic changes, it did not limit mobility to as great an extent as deeper soft tissue injury or joint injury. In the superficial injury group, the arytenoid cartilage maintained its mobility. This is demonstrated by a relatively large change in the length of the distance from the centerline to the vocal process in the neutral position when compared to the length of the same line in the position in full abduction. Also, the maximum glottic area from the experimental side in the superficial injury group most closely approximated the glottic area from the control side. The one exception to this was the group 1 animal that showed reduced mobility on morphometric analysis and had evidence of cartilaginous injury on histologic examination. The experimental injury in this animal was most likely deeper than intended, and if so, would account for the noncharacteristic findings.

Animals in the deep soft tissue injury and joint injury groups demonstrated more limitation in arytenoid mobility than the superficial injury group. The sample size is small and there is considerable overlap, but group 2 showed a 25% to 71% change in glottic area on the experimental side, as compared with group 3, which showed a 66% to 87% change in glottic area on the experimental side. These findings are substantiated by the histologic analysis, which showed loss of the joint space in all of the group 3 animals studied and near–cartilaginous fusion in 2. One animal in group 3 maintained a relatively large glottic area on the experimental side. Histologic analysis, however, demonstrated significant cartilage resorption, as though the animal had undergone a medial arytenoidectomy, and near-fusion of the remaining arytenoid fragment to the cricoid ring. There was minimal, if any, passive movement of the arytenoid on the cricoid at palpation. In the deep soft tissue injury group of animals, the joint space was maintained. A dense fibrous band over the synovial space appeared to limit mobility.

Deep soft tissue injury and joint injury produced clinically significant limitation in arytenoid mobility. In this animal model the degree of limitation of motion was directly dependent on the depth of the initial injury. The injury was reproducible and relatively well tolerated in the canine species. Injury to only one cricoarytenoid joint minimized the potential for airway compromise and provided the opportunity to study the disease process.

This model will allow further study of future methods of diagnosis and treatment. First, since it is difficult to distinguish joint injury, with loss of the joint space and fusion, from a deep soft tissue injury, with maintenance of the joint space, on in vivo palpation, future study needs to be directed at refining radiographic studies to help distinguish fusion from fibrosis. Second, surgical trials comparing the results of posterior cricoid split and cartilage grafting with mucosal grafting need to be undertaken. Finally, the model may be useful in the development of joint replacement modalities that will reproduce normal motion and are similar to orthopedic procedures currently in use for other synovial joints.

CONCLUSIONS

Posterior glottic stenosis is a disabling disease process that is most commonly caused by prolonged endotracheal intubation for mechanical ventilation. The length of intubation prior to the development of PGS is variable. This finding indicates that factors other than length of intubation influence the development of PGS. Because PGS is rare, clinical study is difficult. Therefore, an animal model would be beneficial in improving our general understanding of the histologic changes that create PGS, and it would allow prospective randomized trials of therapeutic interventions.

These experiments demonstrate that it is possible to produce limitation of cricoarytenoid joint mobility in the canine species. Lasers were used to create an injury over the cricoarytenoid joint that simulated an injury that could be produced from exposure to an endotracheal tube. Morphometric analysis of the harvested larynx objectively documented the degree of arytenoid mobility and will be useful in evaluating the results of future therapeutic interventions. The histologic changes that created PGS ranged from fibrosis and scarring over the joint space to loss of the joint space with fusion of the arytenoid and cricoid cartilages. These changes were directly related to the depth of the initial injury. By limiting the injury to one side of the larynx, the current model reliably reproduced mobility limitation with minimal risk of airway compromise. Histologic analysis of the results revealed the range of changes that resulted in limitation of arytenoid mobility. The model is relatively safe and easily reproducible. It will be useful in developing improved methods of diagnosis, staging, and treatment for patients with PGS.

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HEAD AND NECK SURGERY & ONCOLOGY

The 2nd International Symposium in Israel on Head & Neck Surgery and Oncology will be held November 1-5, 1998, in Jerusalem, Israel. For more information, contact Conference Secretariat, CARMEL Organizers of Conferences & Events, PO Box 1912, Ramat Gan 52532, Israel; telephone +972-3-5754040; fax +972-3-5753107; email: tcarmel@netvision.net.il.

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FIRST REPORT OF HÜRTHLE CELL CARCINOMA REVEALED BY OCTREOTIDE SCANNING

CHARLES J. WILSON, MD
KEESLER AFB, MISSISSIPPI

JANET M. WOODROOF, MD
KANSAS CITY, KANSAS

DOUGLAS A. GIROD, MD
KANSAS CITY, KANSAS

Octreotide is an 8-chain amino acid analog of somatostatin. Somatostatin and its receptors occur naturally in multiple sites within the body and serve a suppressive role in endocrine hormone release. When octreotide, which has a considerably longer half-life than somatostatin, is combined with a radioactive isotope, receptor-based imaging can be performed to visualize tumors with high concentrations of somatostatin receptors. Tumors of neural crest origin — pituitary adenomas, islet cell tumors, medullary thyroid carcinomas, pheochromocytomas, carcinoids, and paragangliomas — all express high levels of somatostatin receptors. We present the first reported positive octreotide scan of a Hürthle cell carcinoma of the thyroid and, more important, discuss the role of octreotide scanning in otolaryngology, which has not yet been reviewed by our literature.

KEY WORDS — Hürthle cell carcinoma, octreotide scanning, somatostatin.

CASE REPORT

A 68-year-old man with a 6-month history of a tender, 4 × 4-cm left axillary mass had an excisional biopsy performed at an outside facility, with preliminary pathology suggesting metastatic medullary thyroid carcinoma, although immunostaining for calcitonin was negative. Computed tomography of the neck, chest, and abdomen revealed a partially calcified 6-cm mass involving the left thyroid lobe with no other abnormalities. The patient was then referred to Kansas University Medical Center for definitive therapy.

The patient's past medical history was significant for hypertension, tonsillectomy, adenoidectomy, appendectomy, and cholecystectomy. The patient was a nonsmoker and had no personal or family history of cancer or multiple endocrine neoplasia. Physical examination revealed a large thyroid mass, normal vocal cord function, and no other neck masses. Blood and urine chemistry testing showed normal levels of calcitonin and catecholamines. Nuclear scanning with indium 111–octreotide (Mallinckrodt) was performed to evaluate for other metastatic lesions. The octreotide scan showed abnormal uptake at 4 and 24 hours in the left side of the neck (Fig 1). No other areas of abnormal activity were seen.

The patient was taken to the operating room, where a total thyroidectomy with left modified neck and paratracheal node dissection was performed. The light microscopy, electron microscopy, and immunostaining of the pathology specimen were consistent with a diagnosis of Hürthle cell carcinoma (HCC). The neck dissection and paratracheal node dissection were free of metastasis, with clear margins in the main specimen. Iodine 123 scanning showed a focus of uptake in the thyroid bed, and therefore, the patient received 152 mCi of iodine 131 radioablation to residual thyroid tissue 1 month postoperatively. The patient remains disease-free at 6 months from resection and is being followed up with testing of thyroglobulin levels and repeat octreotide scanning.

DISCUSSION

Octreotide Scintigraphy. Somatostatin is a small regulatory peptide of 14 amino acids that normally serves an inhibitory role in endocrine hormone release in a number of organ systems. Tumor cells of these organ systems and some non-neuroendocrine lesions can express high levels of somatostatin receptors that offer an avenue of localization and possibly therapy. Unfortunately, somatostatin has a half-life of only 2 to 3 minutes, limiting its utility. Octreotide is an analog of somatostatin with a half-life of 90 minutes, allowing for its stable combination with indium 111 and indium 123, and is therefore useful as an imaging tool. Tumors that show uptake of octreotide include those with amine precursor up-

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From the Departments of Otolaryngology–Head and Neck Surgery (Wilson, Girod) and Pathology (Woodroof), Kansas University Medical Center, Kansas City, Kansas. Dr Wilson's address is 81st MSGS/SGCSL, 301 Fisher St, Keesler AFB, MS 39534.

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CORRESPONDENCE — Douglas A. Girod, MD, Dept of Otolaryngology–Head and Neck Surgery, Kansas University Medical Center, 3901 Rainbow Blvd, Kansas City, KS 66160-7380.

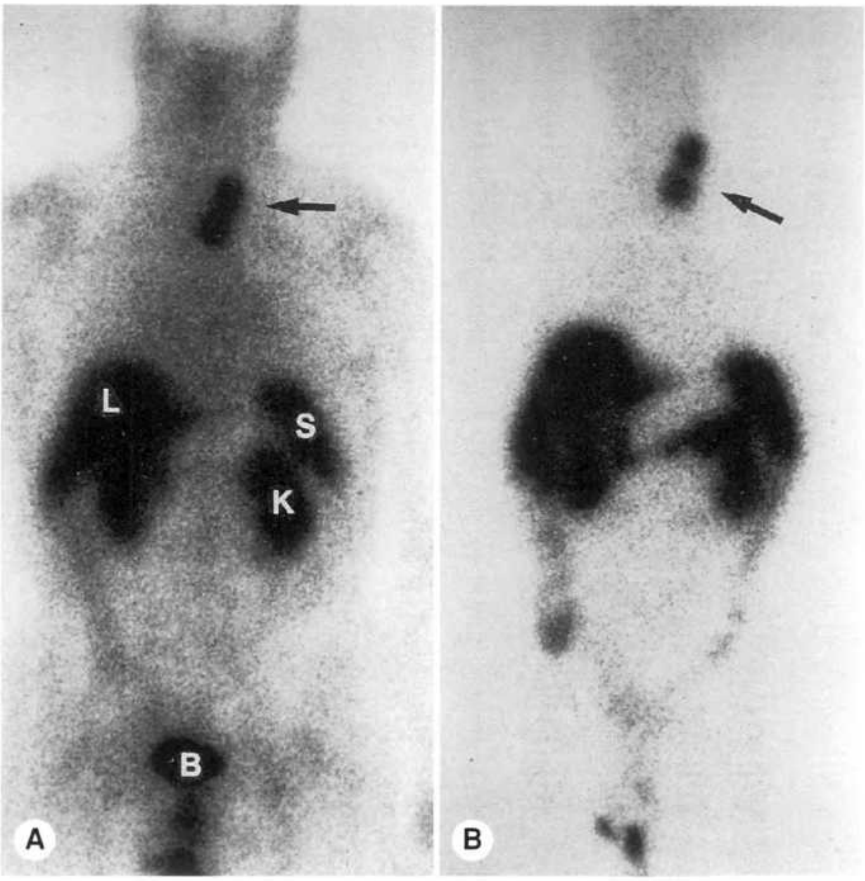

Fig 1. Octreotide scintigraphy. A) Four hours after octreotide injection, showing normal uptake in liver (L) and spleen (S) and excretion via kidney (K) and bladder (B). B) Twenty-four hours after injection, showing persistent abnormal uptake in left side of neck (arrows on A and B).

take and decarboxylation (APUD) characteristics and those of neural crest origin. Specifically, these include both hormone-producing and non–hormone-producing pituitary adenomas, gastrinoma, insulinoma, glucagonoma, paraganglioma, pheochromocytoma, carcinoid, and medullary thyroid carcinoma. However, somatostatin receptors have been demonstrated with various incidence rates in vitro and in vivo on other tumors and lesions such as neuroblastoma, meningioma, renal cell carcinoma, tuberculosis, sarcoidosis, lymphoma, and, now, HCC.[1]

Octreotide Scanning of Hürthle Cell Carcinoma. Hürthle cell carcinoma has been shown to take up the radiotracers of sestamibi and dimercaptosuccinic acid.[2,3] It has not previously been reported to possess somatostatin receptors or show uptake on octreotide scanning. This case report identifies a thyroid malignancy other than medullary thyroid carcinoma that can be identified with octreotide scintigraphy.

The most aggressive of the well-differentiated thyroid malignancies, HCC is regarded as a modified follicular neoplasm and represents 3% to 5% of all thyroid cancers. The incidence of distant metastasis has been reported to be 34%, with a 10-year survival rate of 65%.[4] The treatment of choice for HCC is surgery, as chemotherapy and radioablation are of little benefit.

While HCC does not routinely show uptake of iodine 131, some of these tumors do. Iodine 131 scintigraphy is routinely performed to evaluate for uptake and to eliminate residual thyroid tissue, thereby allowing for postoperative follow-up for recurrences of HCC by nuclear scanning or thyroglobulin level assay.

The histopathology of our patient's neoplasm was consistent with typical descriptions of HCC, being composed of large polygonal cells with pleomorphic nuclei, and granular cytoplasm with abundant mitochondria (Fig 2). Immunohistochemical staining of this specimen was positive for thyroglobulin and negative for calcitonin and chromogranin.

Octreotide in Otolaryngology. The use of octreotide nuclear scanning in otolaryngology has not, to date, been reviewed by the literature in our specialty. There are several areas of potential use. Paragangliomas almost always show uptake on octreotide scanning. Krenning et al[1] showed 100% sensitivity in detection of paraganglioma tumors in their 33 patients, and Hoefnagel[5] showed 97% sensitivity in the 38 patients in his review article. This sensitivity is especially useful in detecting multicentricity of paragangliomas, which occurs at an incidence of 10% in

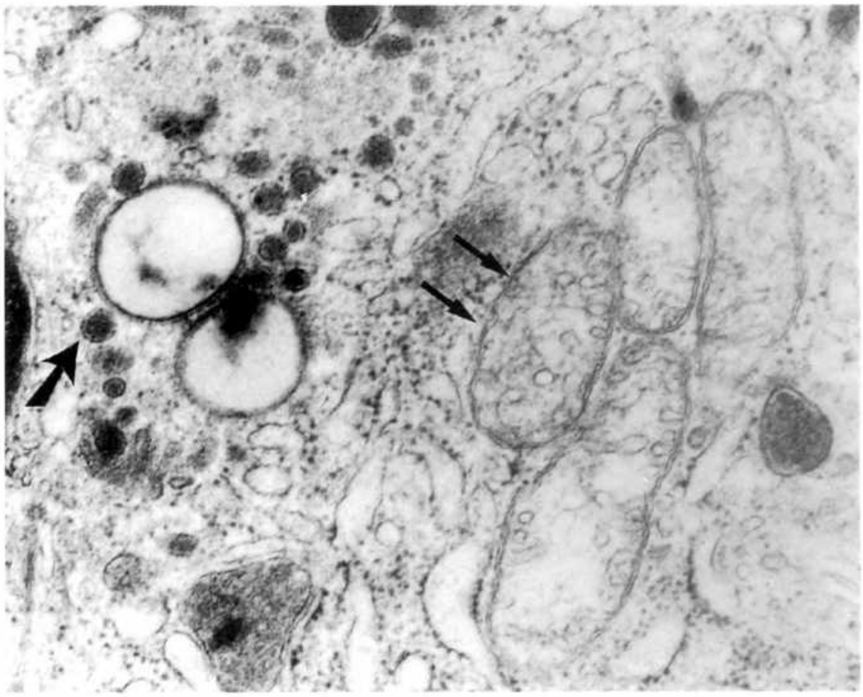

Fig 2. Electron photomicrograph of Hürthle cell carcinoma showing granular cytoplasm, multiple mitochondria (double arrows), and neurosecretory granules (single arrow; original ×28,000).

nonfamilial paragangliomas and an approximate incidence of 35% in familial paragangliomas.[6] Because the familial incidence of paragangliomas is approximately 8%, octreotide scanning can be useful for screening family members.

Octreotide scanning can also be used to evaluate recurrence of tumors that have previously shown uptake and have undergone treatment. Computed tomography and magnetic resonance imaging of recurrences can be difficult in the face of postoperative scarring or postirradiation fibrosis. Octreotide scanning offers the advantage of identifying functioning tumor cells. Some neuroendocrine tumors can be followed up after therapy with serial serum levels of an identifying protein, such as thyroglobulin in medullary thyroid carcinoma or gastrin in gastrinoma. If serum protein levels rise above baseline after therapy, octreotide scanning can be used for localization of recurrences at the primary site or possible sites of metastasis, if other imaging modalities fail to identify tumor.

Lesions occurring in the head and neck that have now shown uptake on octreotide scintigraphy include paraganglioma, meningioma, (now) HCC, medullary thyroid carcinoma, lymphoma, astrocytoma, granulomatous disease, and pituitary adenoma.[5] Another tumor encountered by the head and neck surgeon that may have somatostatin receptors is olfactory neuroblastoma. These tumors, arising in the nasal cavity, are thought to be of neuroendocrine origin and have been associated with hormone-secreting syndromes.[7] To date, there have been no positive or negative reports of octreotide uptake in olfactory neuroblastomas, and further investigation is needed.

CONCLUSIONS

1. Octreotide nuclear scanning can identify neuroendocrine tumors and other lesions having somatostatin receptors.

2. Hürthle cell carcinoma can have somatostatin receptors and be identified with octreotide scintigraphy.

3. Octreotide scanning is useful in otolaryngology for evaluating disease recurrence or metastasis of the head and neck in APUD and neural crest tumors that have shown previous octreotide uptake, identifying multicentricity in paragangliomas, and screening family members for paragangliomas.

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PRIMARY ASPERGILLOSIS OF THE LARYNX AND SQUAMOUS CELL CARCINOMA

LAURENT BEUST, MD

BENOIT GODEY, MD
RÉGIS GROLLIER, MD

FRANÇOIS LE GALL, MD
GUY LE CLECH, MD

RENNES, FRANCE

Primary laryngeal aspergillosis is exceptionally rare, and only five cases have been reported in the literature. We present here two cases of invasive laryngeal aspergillosis without tracheobronchopulmonary extension and without any generalized immune deficit. Aspergillosis developed after local radiotherapy for a squamous cell carcinoma and preceded a recurrence of the tumor. The relationships between the clinical features, the histopathology, and the etiology of laryngeal aspergillosis and squamous cell carcinoma are discussed. We recommend that following treatment of laryngeal aspergillosis, these rare patients should systematically be investigated for a squamous cell carcinoma or a recurrence thereof.

KEY WORDS — aspergillosis, larynx, squamous cell carcinoma.

INTRODUCTION

Aspergillosis is a fungal infection that is frequently responsible for allergic disease and infections of the airway cavities (the sinuses and pulmonary tuberculous cavities). Invasive aspergillosis (pulmonary and multiorgan) is less frequent and usually occurs in the context of immunodeficiency.[1] In this setting, several cases of dissemination of extensive bronchopulmonary aspergillosis to the larynx have been described.[2-4] Isolated, primary laryngeal aspergillosis is an exceptionally rare disease, and only five cases have been reported in the literature.[5-9]

We report on two patients who presented with isolated laryngeal aspergillosis, without any evidence of immunodeficiency on the available immunologic tests, following radiotherapy for squamous cell carcinoma of the larynx and prior to a recurrence of the tumor. We discuss the possible relationships between the aspergillosis and the squamous cell carcinoma.

CASE 1

A 53-year-old man presented with a squamous cell carcinoma of the middle third of the left true vocal cord that was treated by radiotherapy (total dose of 75 Gy). Eight years later, laser treatment was used to treat laryngeal edema without any clinical signs or histologic evidence of recurrence. One year later, he returned because of hoarseness and dyspnea. Direct laryngoscopy showed irregularity of the left true vocal cord and necrosis with exposure of the thyroid cartilage. Histopathologic examination revealed the presence of laryngeal necrosis interspersed with numerous septate, branching, fungal hyphae with regular borders, leading to a diagnosis of invasive aspergillosis, without signs of tumor relapse (Fig 1). There were no clinical signs or laboratory findings to suggest a state of generalized immunodeficiency. There was no evidence of bronchopulmonary aspergillosis on a chest X-ray or on bronchoscopy.

Amphotericin B was given intravenously for 3 weeks. Initially, there was a good response; however, 6 months later, a relapse was noted associated with dyspnea and dysphagia. A granular mass was evident on the larynx, and repeat biopsies showed the persistence of fungal hyphae combined with a well-differentiated squamous cell carcinoma. A total laryngectomy was performed. The patient remains well with no signs of tumor recurrence or aspergillosis with a follow-up of 30 months.

CASE 2

A 64-year-old man presented with a squamous cell carcinoma of the left true vocal cord that was treated by a partial laryngectomy. Ten years later, he presented with a recurrence of the tumor on the false vocal cord that was treated by radiotherapy (70 Gy). Laryngeal stenosis occurred 8 months later. The biopsies revealed lesions consistent with postradiotherapy necrosis extending to the cartilage but without signs of tumor recurrence. Two months later, an endoscopy was performed because of recurrent dyspnea and showed laryngeal edema with subglottic ne-

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From the Departments of Otolaryngology–Head and Neck Surgery (Beust, Godey, Grollier, Le Clech) and Histopathology (Le Gall), Rennes University Hospital, Rennes, France.

CORRESPONDENCE — Benoit Godey, MD, service ORL, CHU de Rennes, 2 rue Henri le Guillou, 35033 Rennes Cedex 9, France.

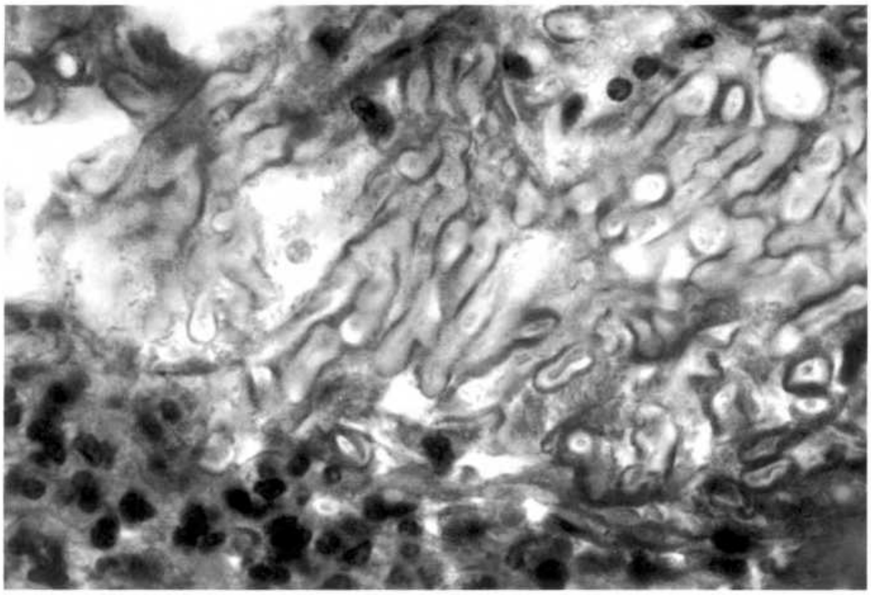


Fig 1. (Case 1) Left true vocal cord biopsy showing numerous septate fungal hyphae, branching at angle of 45°, with regular borders (H & E, original ×1,000).

crosis extending to the cartilage and adherent, necrotic debris over the epiglottis. The biopsies showed the presence of 45° angle, branching, septate hyphae invading the cartilage and a positive Grocott-Gomori stain, leading to a diagnosis of invasive aspergillosis without tumor recurrence (Fig 2). There were no clinical signs or laboratory findings to suggest a state of generalized immunodeficiency. There was no evidence of aspergillosis on a chest X-ray or on bronchoscopy.

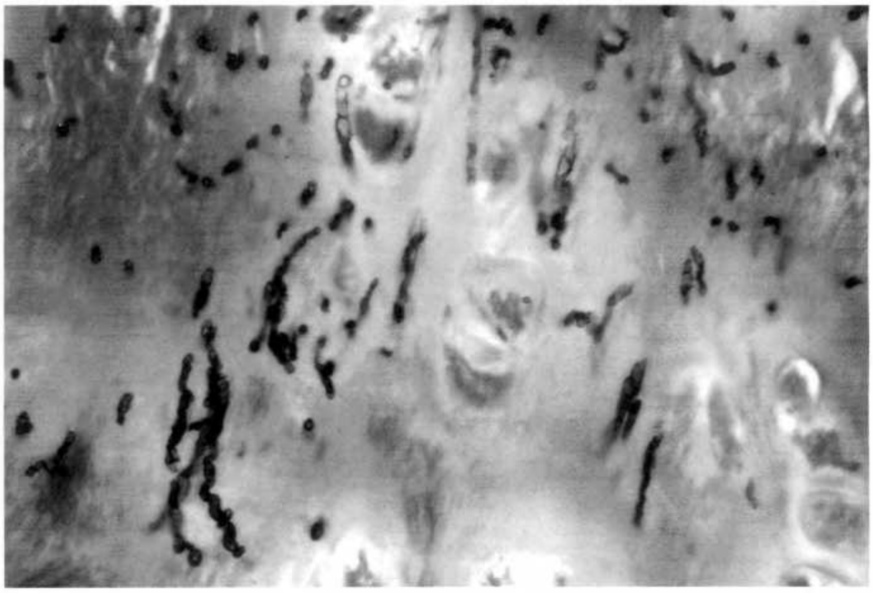
Treatment with intravenous amphotericin B (1 mg/kg per day) was commenced. After 1 week, oral itraconazole (400 mg/d) was given for a further 3 weeks. An endoscopy performed at the end of the treatment demonstrated regression of the necrotic laryngeal lesions. Repeat biopsies revealed relapse of the squamous cell carcinoma, but without evidence of aspergillosis. The patient refused a total laryngectomy. He is currently alive 4 months after the diagnosis of relapse and has a tracheotomy.

DISCUSSION

Laryngeal aspergillosis is very rare. Vrabec[10] has questioned the existence of this entity as a primary disease. He has stated that molds only produce tracheobronchial infection and do not spread to the larynx, in contrast to yeasts (*Candida*, *Cryptococcus*, and *Blastomyces*). So far, eight cases have been published in the literature: five cases of isolated invasive laryngeal aspergillosis[5-9] and three cases of immunodepressed patients with laryngeal extension of tracheobronchial aspergillosis[2-4] who had rapidly progressive disease.

The increased incidence of invasive aspergillosis

Fig 2. (Case 2) Thyroid cartilage biopsy specimen demonstrating deeply invasive fungal organism (Grocott-Gomori stain, original ×400).



in immunodepressed patients is mainly due to pulmonary lesions.[11] The iatrogenic factors responsible are antineoplastic chemotherapy, corticosteroid therapy, radiotherapy, and prolonged antibiotic treatment.[5,6] The main defense against molds is phagocytosis by macrophages and granulocytes; therefore, disorders associated with lymphopenia are less often involved than those associated with neutropenia.[2,8] If the two cases in this report are added to the five in the literature, the seven cases of isolated invasive laryngeal aspergillosis have occurred in six nonimmunodepressed patients and in one patient with steroid-dependent chronic obstructive pulmonary disease.[9] Three patients had cervical irradiation for a laryngeal squamous cell carcinoma. In isolated laryngeal aspergillosis, local factors (radiotherapy, steroid inhalers, and laser treatment) appear to be more important than immunodepression, which contrasts with tracheobronchial aspergillosis with secondary laryngeal extension.

On direct laryngoscopy, the aspergillosis appeared as a whitish ulcer in seven cases, the mobility of the cord was reduced in four cases, and the cartilage was exposed in three cases. These features can also occur in carcinoma.[7,9] Microbiologic examination with culture is the only means by which the species of *Aspergillus* involved can be identified; however, this was not requested following laryngoscopy in any of the seven patients. In one case,[9] cultures performed on tissue from the laryngectomy revealed *Aspergillus fumigatus*. In a retrospective autopsy study, Young et al[12] found a predominance of *Aspergillus flavus* in upper airway lesions. With regard to tracheobronchial aspergillosis with secondary laryngeal involvement, the species identified are *A flavus*[4] and *A fumigatus*.[2]

The diagnosis requires histopathologic examination. Invasion of the epithelium by fungal filaments leads to tissue necrosis characterized by an adherent creamy coating.[5,6] The overlying epithelium shows acanthosis and pseudoepitheliomatous hyperplasia that can be mistaken for a carcinoma.[7,9] If fungal hyphae are not seen, the presence of a granulomatous reaction and the rarity of mitoses and nuclear atypia indicate the need for special stains (periodic acid–Schiff and Grocott-Gomori) to be performed. This allows visualization of the hyphae, and the presence of 45° angle, branching, septate hyphae supports the diagnosis of aspergillosis.[6,9] The extensive necrosis of the epithelium due to vascular, mycelial emboli can mask a tumor recurrence by altering the mucosa and the vessels of the laryngeal epithelium. This was probably the case in our two patients, in whom it was not possible to document recurrence of the carcinoma following treatment of the aspergillosis. The subsequent appearance of the carcinoma after a short delay suggests that the tumor relapse was present at the same time as the mycosis.

The possible carcinogenicity of *Aspergillus* needs to be considered. *Aspergillus* produces carcinogenic compounds (aflatoxin and sterigmatocystines) known to play a role in squamous cell carcinoma of the lung and hepatocellular carcinoma by mutating codon 249 of the p53 protein.[13] However, a direct role for *Aspergillus* in these tumors has not been proven.

In the literature, all the patients were successfully treated.[5-9] The various local measures taken were microsurgical resection of the lesions,[5,6] extensive surgical excision,[9] and local application of nystatin.[7] In view of the invasive nature of these mycoses, we believe that systemic antifungal therapy is justified. Such treatment is necessary in order to prevent progression to invasive aspergillosis (pulmonary and multiorgan) and because an underlying squamous cell carcinoma can only be identified once the mycosis has been treated. The drug of choice is intravenous amphotericin B.[2,3,9] The recent introduction of itraconazole allows oral treatment, with reduced toxicity but the same therapeutic effect.

Although laryngeal aspergillosis is rare, it must be considered in the differential diagnosis of laryngeal ulceration. There are several aspects to the interrelationship between laryngeal aspergillosis and squamous cell carcinoma: 1) the clinical features and the findings on direct laryngoscopy can both be suggestive of squamous cell carcinoma; 2) etiologically, laryngeal radiotherapy appears to be a factor predisposing to aspergillosis and, in addition, the role of mycotoxins in the development of squamous cell carcinoma needs to be considered; and 3) in terms of the histopathology, aspergillosis can both simulate and mask squamous cell carcinoma. Following treatment of these rare cases of laryngeal aspergillosis, we recommend systematically investigating for a squamous cell carcinoma or a tumor relapse.

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OBSERVATIONS OF LARYNGEAL LESIONS WITH A RHINOLARYNX ELECTRONIC VIDEOENDOSCOPE SYSTEM AND DIGITAL IMAGE PROCESSING

MASAHIRO KAWAIDA, MD
HIROYUKI FUKUDA, MD
NAOYUKI KOHNO, MD
TOKYO, JAPAN

Laryngeal lesions were observed with the Olympus ENF-200 rhinolarynx electronic videoendoscope attached to the Olympus EVIS-200 system, an electronic videoendoscope system of Olympus Optical Co, Ltd. The electronic videoendoscope differs from conventional flexible fiberscopes in having a small light-sensitive charge-coupled device (CCD) chip built into the tip of the endoscope portion. This model has a small CCD chip in its 5-mm–outer diameter tip, and can be introduced into the laryngeal cavity by inserting it through the nasal passages. Dynamic color images obtained by this system were very clear and provided excellent resolution. This electronic videoendoscope system could also be connected to the Olympus EVIP-230 digital image processor, which was compatible with this system. Structure enhancement and color enhancement were performed by processing images. In this paper, the ordinary images and the processed images are described and discussed from the standpoint of diagnostic usefulness.

KEY WORDS — digital image processing, electronic videoendoscope system, laryngeal lesions, laryngoendoscope, rhinolarynx electronic videoendoscope.

INTRODUCTION

It is now possible to apply, to the field of laryngology, electronic videoendoscope systems in which a small charge-coupled device (CCD) chip is built into the tip of the endoscope. The images obtained are converted into electric signals and transmitted, and clear dynamic color images are reproduced on a color video monitor through a video system center.[1,2] The Olympus Optical Co, Ltd, has produced an electronic videoendoscope system, Endoscopic Video Information System 200 (EVIS-200).[3] One of the new models in this product line is the Olympus ENF-200 rhinolarynx electronic videoendoscope, which contains the tip and the insertion tube of the endoscope in a 5-mm outer diameter. Observations and recordings of laryngeal lesions have been made with the system using this new model of the endoscope portion. Since it is also possible to connect a digital image processor capable of processing dynamic images in real time to this system, an attempt has also been made to process the dynamic color images of laryngeal lesions obtained by the rhinolarynx electronic videoendoscope system with this image processor. The images of laryngeal diseases obtained by this system and the processed images are described and discussed from the standpoint of diagnostic usefulness.

EQUIPMENT AND METHODS

Electronic Videoendoscope System. The electronic videoendoscope system used in this study was the EVIS-200 system produced by Olympus Optical Co, Ltd. This system consists of an endoscope portion, a video system center, a xenon light source, a color video monitor, a videotape recorder (VTR), and a color video printer. The endoscope portion used to observe laryngeal lesions is the Olympus ENF-200 rhinolarynx electronic videoendoscope (Fig 1A). The tip and insertion tube of the endoscope have an outer diameter of 5 mm, and the endoscope is capable of being inserted into the laryngeal cavity through the patient's nasal passages (Fig 1B). The specifications of this endoscope portion are shown in the Table. The EVIS CV-200 video system center and the EVIS CLV-U20D light source are used, both connected with the MD-149 videoscope cable. A Sony PVM-1442Q color video monitor, a Sony S-VHS SVO-9500MD VTR, and a Sony UP-5200 color video printer were also used in this study. An external view of the entire system is shown in Fig 2A. The single-plate RGB (red-green-blue) surface scanning method is used in the EVIS-200 and is described below.

A small monochrome CCD chip is built into the tip of the endoscope portion as an ultraminiature video camera. A monochrome CCD chip can only provide black-and-white signals. To generate the 3 primary colors red, green, and blue, the white light from the xenon light source is passed through a high-speed rotating filter in the video system center and sequen-

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From the Department of Otolaryngology, Tokyo Metropolitan Ohtsuka Hospital (Kawaida), the Department of Otolaryngology, Keio University School of Medicine (Fukuda), and the Department of Otolaryngology, Juntendo University School of Medicine (Kohno), Tokyo, Japan.

CORRESPONDENCE — Masahiro Kawaida, MD, Dept of Otolaryngology, Tokyo Metropolitan Ohtsuka Hospital, 2-8-1, Minamiohtsuka, Toshima-ku, Tokyo 170, Japan.

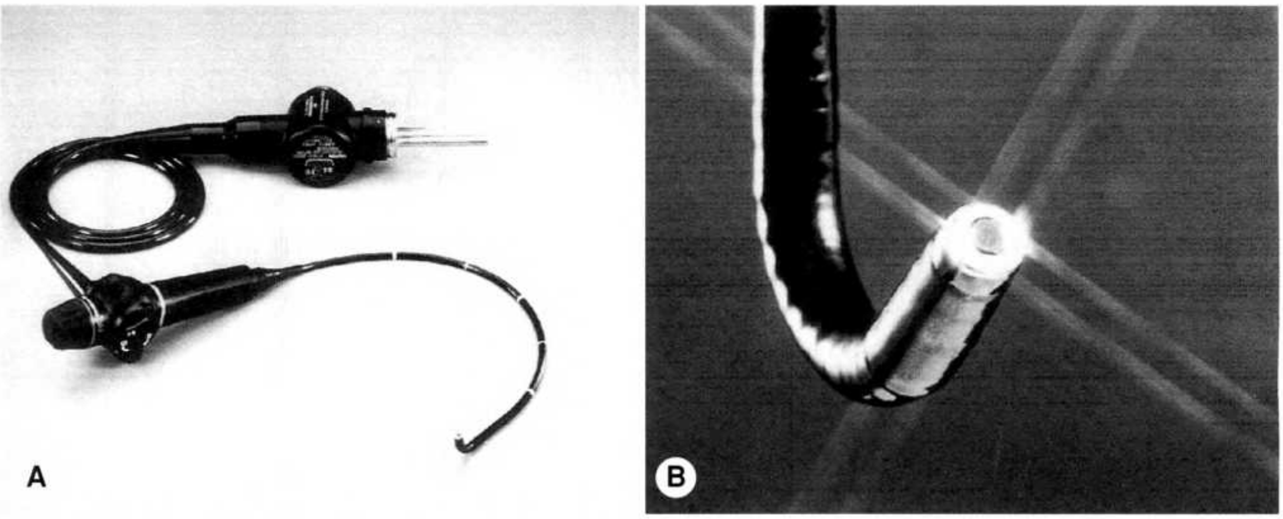


Fig 1. Rhinolarynx electronic videoendoscope Olympus ENF-200. A) External view of entire endoscope portion. B) Tip of endoscope portion is 5 mm.

tially illuminates the target in order. The RGB light components reflected from the target and perceived sequentially by the monochrome CCD chip are converted into electric signals and transmitted to the 3 image memories in the video system center through the electric cable inside the endoscope portion. The dynamic color image is then reconstructed and projected onto the screen of the color video monitor. This image is also recorded on videotape by the VTR. Then, patient information such as name, age, sex, identity number, and endoscopic findings can also be simultaneously input onto the screen of the color video monitor by operating a keyboard.

Digital Image Processor. Image processing of dynamic color images of the laryngeal lesions in patients was attempted by using an Olympus Endoprocessor EVIP-230 digital image processor, which is capable of being connected to the EVIS-200 (Fig 2B). Electric signals from the video system center are input into this device, and digital image processing is performed in real time. It is possible to change an ordinary dynamic image to a processing dynamic image with this device in real time by operating a foot switch. Two types of image processing, structure enhancement and color enhancement, can be performed with this device. An adaptive band enhancement system has been adopted for structure enhancement, and a saturation enhancement system for the red color of hemoglobin has been adopted for color enhancement. Each enhancement system is divided into 7 grades and processed. Moreover, it is also possible to set the mixed mode, which combines the 2 enhancement systems in each grade. In this study, digital image processing is attempted by setting the mixed mode at the maximum value of grade 7 for structure enhancement and grade 3 for color enhancement.

SPECIFICATIONS OF RHINOLARYNX ELECTRONIC VIDEOENDOSCOPE OLYMPUS ENF-200 (OLYMPUS OPTICAL CO, LTD)

Optical system	Field of view	100°
	Direction of view	0° (Forward viewing)
	Depth of field	5 to 50 mm
	Illumination	Light guide system
Bending section	Range of tip bending	Up 130°, down 130°
Distal end	Outer diameter	5.0 mm
Insertion tube	Outer diameter	4.9 mm
Working length		365 mm

Examination Method. In performing endoscopic examinations with this system, surface anesthesia of the patient's nasal and oral cavities is performed in advance by spraying the cavities with 4% lidocaine hydrochloride solution. The patient is examined in the seated position. The insertion tube of the endoscope is later introduced through the nasal passages, and the examiner conducts the endoscopic examination while observing the dynamic image on the color video monitor (Fig 3). The maneuverability of this endoscope portion is almost the same as that of conventional rhinolarynx flexible fiberscopes. The endoscopic findings of laryngeal lesions observed by this system are recorded by the VTR along with the ordinary dynamic image and the processed dynamic image in real time. Still color images are also printed on the color video printer.

SUBJECTS

Two patients are the subjects in this study: a 45-year-old man with a right vocal fold polyp (patient

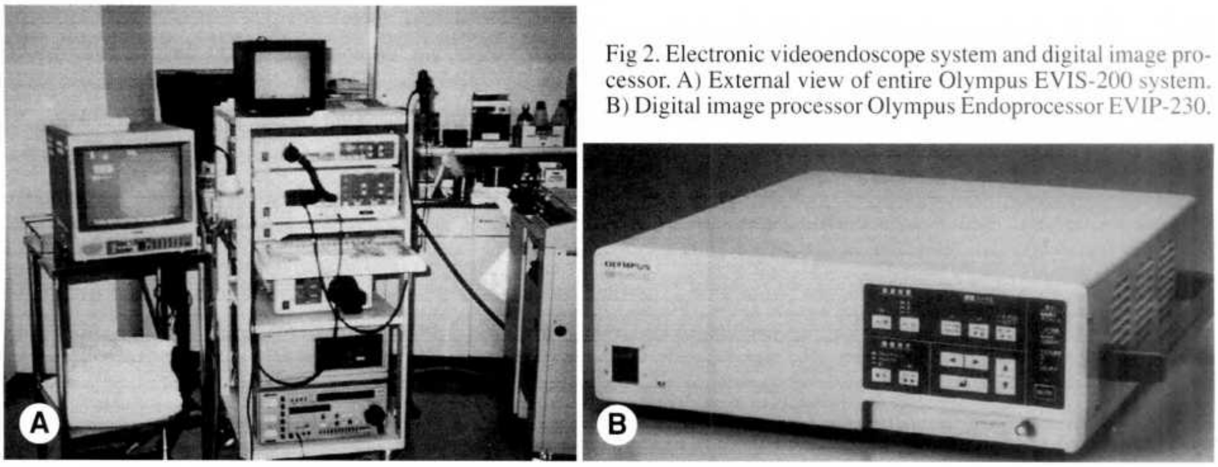


Fig 2. Electronic videoendoscope system and digital image processor. A) External view of entire Olympus EVIS-200 system. B) Digital image processor Olympus Endoprocessor EVIP-230.

1) and a 64-year-old man with right invasive supraglottic cancer (patient 2). Endolaryngeal microscopic laryngosurgery using a direct laryngoscope was performed under general anesthesia in both patients, and the resected lesions were examined histopathologically. The histopathologic diagnoses were benign angiectatic polyp in patient 1, and moderately differentiated squamous cell carcinoma in patient 2.

RESULTS

Even the ordinary image of the laryngeal findings in patient 1 observed with the rhinolarynx electronic videoendoscope system provided a clear image with excellent resolution (Fig 4A). On the other hand, blood vessels coursing in the mucous membrane of the glottis and supraglottis were shown more distinctly by color enhancement, and the path of the blood vessels was very clear. Structure enhancement was simultaneously performed by the digital image processor, but there was hardly any difference between the ordinary image and the processed image in terms of the surface structure of the laryngeal mucous membrane containing the lesion (Fig 4B).

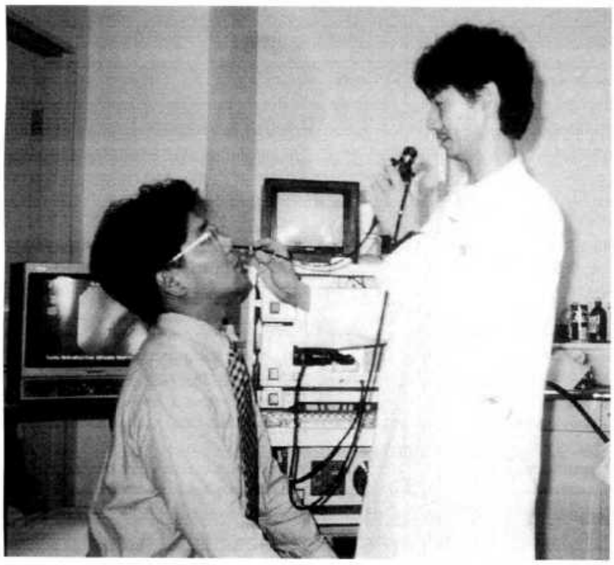


Fig 3. View of actual performance of endoscopic examination with rhinolarynx electronic videoendoscope. Patient is examined in seated position. Flexible insertion tube of endoscope has been inserted through patient's nasal cavity after application of surface anesthesia.

In patient 2, the tumorous lesion was limited to the right supraglottic area alone, and there did not appear to be any abnormal findings in the left supraglottic area on the ordinary image (Fig 5A). The processed image clearly depicted the course of the blood vessels by color enhancement, and the enhanced surface roughness of the tumorous lesion was clearly shown by structure enhancement (Fig 5B). However, the surface rough structure was markedly enhanced in the ventricular fold of the left supraglottic area on the processed image. A biopsy during surgery was performed later in this patient, and invasion by squamous cell carcinoma was histopathologically diagnosed at the site of the surface rough structure in the left supraglottic area as well.

DISCUSSION

An electronic videoendoscope system was developed in which a small CCD chip was built into the tip of the endoscope portion as an ultraminiature video camera. The images obtained from the target were converted into electric signals and transmitted to the video system center, and clear dynamic color images were reconstructed and projected onto the screen of the color video monitor. Therefore, the problem of obtaining a honeycomb image with video laryngoscopy, as when using a conventional flexible fiberscope, is avoided with this system. However, since the tips and insertion tubes of the endoscope were fairly thick in the beginning, the system was first used in the gastrointestinal tract.[3-6] Later, when a smaller CCD chip was developed, it became possible to decrease the outer diameter of the tip and insertion tube of the endoscope, making it possible to use it in the field of bronchology.[7] In 1993, a rhinolarynx

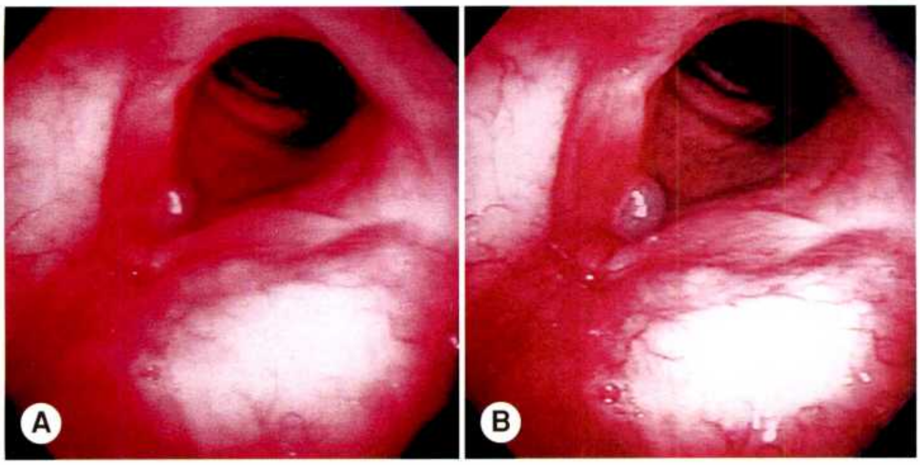


Fig 4. (Patient 1) Laryngeal findings observed by rhinolarynx electronic videoendoscope system in patient with right vocal fold polyp. A) Ordinary image. Reddish polyp is seen in center of membranous portion of right vocal fold. B) Processed image. Course of blood vessels is clearly depicted by color enhancement, but they are hardly detectable by structure enhancement.

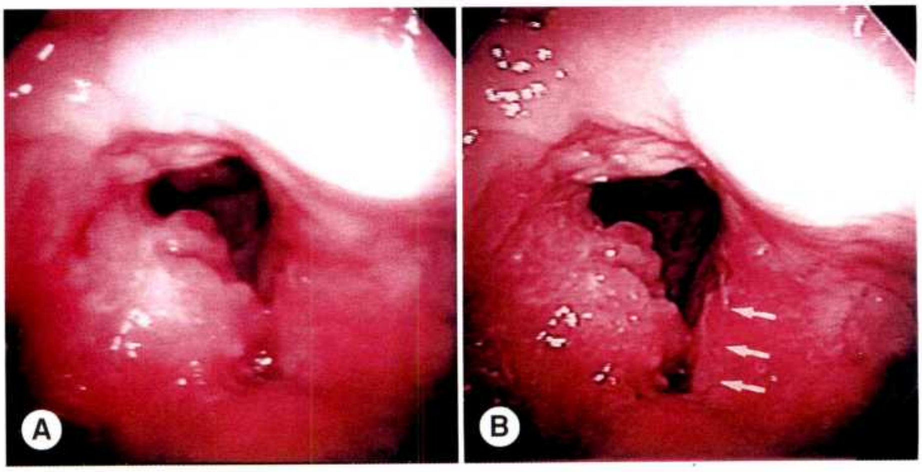
electronic videoendoscope with an approximately 5-mm–outer diameter tip and insertion tube that allowed observations through the patient's nasal passages was marketed by Asahi Optical Co, Ltd. The authors observed laryngeal lesions with a system using this model of the endoscope portion and reported obtaining clear dynamic images with better resolution than the dynamic images observed with conventional rhinolarynx flexible fiberscopes.[1]

The EVIS-200 series was marketed by the Olympus Optical Co, Ltd, as a single-plate RGB surface scanning method electronic videoendoscope system, and for the most part it has been used in the gastrointestinal tract.[3] Recently, the same manufacturer has developed and marketed the rhinolarynx electronic videoendoscope ENF-200 as one of the new models in its EVIS-200 series. Observations and recordings of laryngeal lesions were performed with this system using the ENF-200 in this study. The laryngeal findings obtained with the electronic videoendoscope system were clear and provided superior color reproduction even with ordinary dynamic images.

In addition, there are two distinct methods by which dynamic color images are obtained with this system. One is the single-plate simultaneous color chip method using a color CCD chip, the other the single-plate RGB surface scanning method using a monochrome CCD chip. The color CCD chip contains color pixels and can simultaneously detect different color wavelengths of light with a shutter speed of 0.017 second. Because the monochrome CCD chip must take the 3 primary colors sequentially, this chip requires a shutter speed of 0.05 second, which is 3 times slower than that of a color CCD chip. With the single-plate RGB surface scanning method, the triple color coding function is carried out sequentially by a single picture element housed within the monochrome CCD chip. As a result, the overall size of the television camera built into the tip of the endoscope portion can be made much smaller than that of a color CCD chip. Although the color CCD chip simplifies color acquisition, this chip is considerably larger than the monochrome CCD chip in the electronic videoendoscope system, such that the outer diameter of the tip of the endoscope portion is necessarily wider. This is one reason for a single-plate RGB surface scanning method using a monochrome CCD chip being preferred in the field of laryngology.

On the other hand, images perceived from the tar-

Fig 5. (Patient 2) Laryngeal findings observed by rhinolarynx electronic videoendoscope system in patient with right supraglottic cancer. A) Ordinary image. Tumor is present in right supraglottic area, but there do not be appear to be any abnormal findings in left supraglottic area. B) Processed image. Surface roughness of tumorous lesion is enhanced by structure enhancement. Surface roughness of left supraglottic area is also enhanced (arrows).



get by the CCD chip built into the tip of the endoscope portion are transmitted in the form of electric signals to the video system center. It has been reported that it is possible to directly input the electric signals into the personal computer and perform image processing of the images obtained by the electronic videoendoscope system.[8] Since special systems containing hardware and software had not yet been developed, it had the disadvantage of requiring a considerable amount of time for the processed image to be visualized. A special processing integrated circuit is now included in the EVIP-230, developed and marketed by Olympus Optical Co, Ltd, in 1995, making it possible to connect it to the EVIS-200 system and to process images in real time. Therefore, laryngeal lesions were observed with the EVIS-200 system by using the rhinolarynx endoscope portion of the ENF-200, and real time digital image processing by the EVIP-230 was also performed. An adaptive band enhancement system containing band pass filtering has been adopted for structure enhancement in the EVIP-230, and repeated transformation of the coordinates of the electric signals enables enhancement of the contours and surface roughness of lesions. Color saturation enhancement, which especially enhances the red color of hemoglobin, has been adopted for color enhancement, and the goal of so doing was to detect only cancers containing a great deal of blood and the reddening caused by inflammation. Use of the foot switch makes it possible to change over from ordinary images to processed images in real time.

The course of blood vessels in the laryngeal mucous membrane was very clearly depicted by color enhancement in patient 1, who had a left vocal fold polyp. On the other hand, even when the highest grade of structure enhancement was performed, no surface roughness was visible on the lesion or the other mucous membrane. Thus, it can be concluded that the surface of benign vocal fold polyps and normal laryngeal mucous membrane is extremely flat. In patient 2, who had a right supraglottic cancer, the course of blood vessels was depicted by color enhancement, and the marked surface roughness of the tumorous lesion, which was located in the right supraglottic area, was clearly depicted by structure enhancement. The same structure enhancement also revealed surface roughness in the left supraglottic area. Later, a biopsy was performed during surgery, and histopathologic examination revealed cancer invasion of the left supraglottic area as well. Thus, it could be said that the enhanced surface roughness matched the site of cancer invasion. Accordingly, it was possible to predict contralateral invasion at the stage of the endoscopic examination on an outpatient basis.

On the basis of the above, the electronic videoendoscope system using a rhinolarynx endoscope portion appears to be extremely useful for endoscopically diagnosing laryngeal cancer. Moreover, it is possible to predict the site of cancer invasion with a certain degree of accuracy by using the digital image processor in combination, and this provides valuable information for choosing optimal treatment. In the future, we plan to continue our assessment by increasing the number of patients.

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EXTRACORPOREAL SHOCK WAVE LITHOTRIPSY OF PAROTID STONES

RESULTS OF A PROSPECTIVE CLINICAL TRIAL

HEINRICH IRO, MD
HOMBURG, GERMANY

JOHANNES ZENK, MD
HOMBURG, GERMANY

FRANK WALDFAHRER, MD
HOMBURG, GERMANY

WOLF BENZEL, MD
ERLANGEN, GERMANY

THOMAS SCHNEIDER, MD
ERLANGEN, GERMANY

CHRISTIAN ELL, MD
WIESBADEN, GERMANY

The extracorporeal shock wave treatment of parotid stones is a rather new therapy. Its usefulness was determined in a prospective study. Seventy-six patients (36 female, 40 male, 2 to 80 years of age) with symptomatic, sonographically detectable solitary sialoliths of the parotid gland were treated with an extracorporeal piezoelectric shock wave therapy after unsuccessful conservative therapy (sialagogues, gland massage, bougienage of the secretory duct). At most, 3 treatments per patient were performed. Altogether, 38 of the 76 patients (50%) were free of stones and no longer suffered from complaints after completion of shock wave treatment and a mean follow-up period of 48 months (range 6 to 71 months). During the follow-up period, in no case could renewed stone formation be observed. Residual stone fragments were detectable in 20 patients (26%), but did not cause further symptoms. Thirteen patients (17%) with residual stone fragments stated a significant improvement of their complaints after therapy. Five patients (7%) did not observe any changes of their pretherapeutic complaints and underwent parotidectomy. The therapeutic success was not influenced by stone size or by stone localization within the gland. During the follow-up period, no side effects of the therapy were identified. With stones of the parotid gland, extracorporeal shock wave lithotripsy is — after one has used conservative therapies (sialagogues, gland massage) — the treatment of choice, avoiding in the majority of cases a parotidectomy with its operative risks (paresis of the facial nerve, Frey's syndrome).

KEY WORDS — lithotripsy, salivary glands, shock waves, sialolithiasis.

INTRODUCTION

The frequency of stones occurring within the large salivary glands of the head is estimated at 1.2%.[1] If a stone blocks the secretory duct of a salivary gland, this will cause a saliva congestion in combination with a painful sialadenitis.[2] The formation of stones in the large salivary glands of the head occurs in 80% of the cases in the submandibular gland and only in 20% in the parotid gland.[3] These differences in lithogenesis are attributed to anatomic factors and to the differences in saliva composition between these 2 glands.[4,5] If it proves impossible to wash out the sialolith by stimulating increased salivation through doses of sialagogues in combination with gland massage and, if necessary, duct bougienage, the remaining therapeutic methods comprise duct slitting, extracorporeal shock wave lithotripsy, and the extirpation of the affected gland. While opening the secretory duct of the submandibular gland poses no problems up to the hilus area, slitting the secretory duct of the parotid gland is considered dangerous because of possible stenosis. In 1989, we reported on the first successful extracorporeal shock wave therapy of a sialolith[6]; other reports followed.[7-13] So far, however, there are no evaluations of large groups of patients followed up for a reasonable period of time. In this study, we report our experience with extracorporeal shock wave therapy applied to 76 patients with solitary stones of the parotid gland.

PATIENTS AND METHODS

Patients. From January 1990 until December 1995, altogether, 109 patients with stones in the parotid glands were submitted to the Department of Otorhinolaryngology–Head and Neck Surgery at the University of Erlangen-Nuremberg. Subsequent to the anamnesis and clinical examination, sonography of the affected gland was performed (Sonoline SI 450, Siemens Company, Germany). Extracorporeal shock wave therapy was indicated within the framework of a prospective study and according to defined criteria of inclusion and exclusion (Table 1).

In 8 of the 109 patients, multiple concretions could

From the Department of Otorhinolaryngology–Head and Neck Surgery, Saarland University, Homburg (Iro, Zenk, Waldfahrer), the Department of Otorhinolaryngology–Head and Neck Surgery (Benzel) and the Department of Medicine I (Schneider), University of Erlangen-Nuremberg, Erlangen, and the Department of Medicine II, Dr Horst-Schmidt-Kliniken, Wiesbaden (Ell), Germany.

CORRESPONDENCE — Heinrich Iro, MD, Dept of Otorhinolaryngology–Head and Neck Surgery, Saarland University, D-66421 Homburg/Saar, Germany.

TABLE 1. CRITERIA OF INCLUSION AND EXCLUSION FOR SHOCK WAVE THERAPY

Criteria of Inclusion	*Criteria of Exclusion*
Symptomatic disease	Duct slitting or duct stenosis
At least 1 purulent parotitis in history	Impaired blood coagulation Cervical spine problems with respect to posture during therapy
No recovery after 3 months of conservative therapy	
Exact sonographic localization of solitary concretion	Multiple concretions

be identified in the parotid gland affected. Further, 25 of the 109 patients were free of symptoms and stones after 3 months of conservative therapy (gland massage, duct bougienage, sialagogues), so that no additional measures were required.

Seventy-six of the 109 patients with stones of the parotid gland fulfilled all criteria of inclusion and no criteria of exclusion.

The ages of the 76 patients in the study (36 female, 40 male) ranged from 2 to 80 years; the average age was 50 years. The mean duration of complaints on the first appearance at the department was 56 months (range 1 to 240 months). The diameters of the stones sonographically detected ranged from 2.5 to 12.0 mm (mean 5.9 mm).

Fifty-seven (75%) stones were found in the distal part of the parotid duct, and 19 (25%) could be localized sonographically within the intraparenchymatous duct system.

Shock Wave Generator. For shock wave generation, the piezoelectric lithotriptor Piezolith 2500 (R. Wolf Company, Knittlingen, Germany) was used. The technical data were already published in the course of the first clinical trial.[13]

Preparation for Treatment. Before, 1 hour after, and 24 hours after shock wave lithotripsy, B-scan sonography of the affected gland was carried out. In addition, before and 24 hours after treatment, all patients were submitted to a hearing test (pure tone audiogram) in order to detect possible hearing damage caused by the therapy. There was no premedication prior to the application of shock waves.

Treatment. With the exception of a 2-year-old boy who was treated under general anesthesia, the shock waves were applied without any sedation or analgesia. Before treatment, the stone was first sought on 2 levels with the integrated B-mode scanner (7.5 MHz) of the lithotriptor and then focused. The most suitable angle for treatment with the best visibility could be chosen freely. During 1 treatment session a maximum of 3,000 shock waves was applied (frequency 2.5 Hz, changing of intensity after each 1,000 shock waves, maximum focus pressure 80 megapascals). The treatment was cut off prematurely if the stone could no longer be localized sonographically or if increasing unrest of the patient seemed to prevent an ordered application of shock waves.

All patients were first subjected to shock wave lithotripsy and then to auxiliary measures (gland massage for steady salivation, duct bougienage, stone extraction basket). Prophylactically, all patients were orally administered an antibiotic (roxithromycin) and an antiphlogistic (diclofenac) for 3 days.

Regular examinations after the last shock wave treatment took place every 2 months and, after 1 year, every 6 months.

If residual concretions were sonographically detectable, a second or third treatment was carried out 2 to 4 months after the first one. If the purpose of the therapy — the patients being free of stones — was attained, no further shock wave sessions were added. The maximum number of treatments amounted to 3 sessions. The mean follow-up period was 48 months (range 6 to 71 months).

RESULTS

Altogether, the 76 patients received 162 shock wave treatments. The results of extracorporeal shock wave lithotripsy are listed in Table 2. The results of all patients (n = 76) considered, after termination of the study 38 patients (50%) were free of stones. In 33 patients (43%), residual sialoliths were sono-

TABLE 2. RESULTS OF EXTRACORPOREAL SHOCK WAVE TREATMENT IN 76 PATIENTS

Treatment	*Free of Stones and Symptoms*	*Free of Complaints With Residual Fragments*	*Residual Fragments With Improvement of Complaints*	*No Change in Complaints*
First (n = 76)	28/76	11/76	35/76	2/76*
Second (n = 46)	6/46	16/46	21/46	3/46
Third (n = 40)	4/40	20/40	13/40	3/40
Total (N = 76)	38/76 (50%)	20/76 (26%)	13/76 (17%)	5/76 (7%)

*Parotidectomy in external departments.

TABLE 3. RESULTS IN CORRELATION WITH AVERAGE STONE SIZE AND STONE LOCALIZATION

	Free of Stones and Symptoms (n = 38)	*Free of Complaints With Residual Fragments (n = 20)*	*Residual Fragments With Improvement of Complaints (n = 13)*	*No Change in Complaints (n = 5)*
Average stone size* (mm)	6.2 ± 2.1	5.5 ± 1.9	5.7 ± 2.4	5.5 ± 2.0
Distal localization (duct and hilum)	31/57	16/57	9/57	1/57
Proximal localization (parenchyma)	7/19	4/19	4/19	4/19

*Mean ± SD.

graphically detectable; 20 of these patients did not report any complaints on successive follow-up examinations.

The 5 patients in whom the extracorporeal shock wave lithotripsy had not caused any change in the preexistent complaints submitted thereafter to a parotidectomy. Two of these 5 patients had already undergone surgery in external departments after the first shock wave treatment, however.

Neither the factor "free of stones" nor the persistence of complaints was influenced by stone localization within the gland or by stone size (Table 3).

As an acute effect of therapy, the patients felt a slight shooting pain on the skin surface above the salivary gland treated. These complaints were considered by the patients to be minor.

None of the patients suffered from a clinically detectable impairment of the facial nerve. In 40% of the cases (65 shock wave treatments), minor bleeding from the parotid duct and easily reversible petechial bleedings without permanent consequences occurred. An impairment of teeth or tooth fillings was not detected.

In 70% of all patients, a slight swelling of the gland, lasting at most 24 hours, developed immediately after the shock wave therapy. In 2 cases, after the therapy, a bacterial parotitis developed that was controlled by intravenously administered antibiotics (ampicillin-sulbactam combination). Audiometric measurements did not detect any case of hearing loss due to therapy.

None of the 76 patients missed the follow-up examinations. During the whole follow-up period, none of the patients free of stones suffered a recurrence. Further, the patients with residual concretions reported having no symptoms.

DISCUSSION

The introduction and establishment of extracorporeal lithotripsy of sialoliths has fundamentally changed the guidelines for sialolith treatment. Until a few years ago, all 76 patients treated in this study would have been submitted to a parotidectomy. By means of extracorporeal shock wave therapy, 50% of these cases were completely freed of stones and a further 20 patients (26%) were freed of complaints in spite of detectable residual fragments. Although the results we had achieved with a significantly smaller group of patients (n = 16),[13] of whom 81% were freed of stones, were not reached in this study, the value of extracorporeal lithotripsy for the treatment of sialoliths is confirmed. If intensive conservative measures like duct bougienage, gland massage, and sialagogues have failed to eliminate the stone, we consider the shock wave treatment to be the method of choice, avoiding in nearly all patients an operative removal of the parotid gland affected with sialolithiasis. This view is shared by other authors who, after extracorporeal shock wave therapy of sialoliths in considerably smaller groups of patients, report that 53% to 80% of patients are free of stones or complaints.[7-12]

All in all, shock wave application in cases of sialolithiasis of the parotid gland can be considered as having few side effects. After only 2 of the 162 treatments, a bacterial parotitis developed. In the framework of this study, neither a shock wave–related lesion of the auditory system nor an irritation of the facial nerve could be identified. In addition, shock wave therapy for stones of the parotid gland is usually feasible without the administration of sedation or analgesia. In contrast, an operative stone removal (parotidectomy) always requires general anesthesia. Further, the operative procedure has the not-insignificant risks of (in most cases transient) paresis of the facial nerve and of the subjectively very unpleasant gustatory sweating (Frey's syndrome).[14]

Some aspects of lithotripsy deserve special attention. In none of our patients who became free of stones by means of shock wave therapy, during the follow-up period of up to 6 years, was a recurrence of stones identified. This is quite remarkable, since extracorporeal shock wave therapy does not aim at the cause of the stone disorder, ie, the dyschylia, but only at the resulting concretion and symptoms. Due to short follow-up periods, there are no reports on that subject by other authors.

While being free of stones can be considered the sole criterion for a successful therapy, it is currently not clear if the lack of complaints in spite of identifiable residual calculi is of lasting use to the patient. Lack of complaints with residual stone fragments after lithotripsy certainly results from the fact that the saliva forces a course through the fragmented stone pieces to the ostium. During the follow-up period, we did not observe, in our symptom-free patients, any sticking together of the fragments leading to a renewed occurrence of the symptoms of obstructive sialadenitis. This observation was also reported by other authors.[11]

The most probable explanation of why, in spite of fragmentation, some patients could become free of stones while others could not is that the different concretions were not impacted with the same firmness in the secretory ducts. Mueller et al[15] reported that extracorporeal shock wave lithotripsy of renal pelvic stones is more successful than the treatment of ureteral calculi and explained this phenomenon by the fact that the stones in the ureter are quite often impacted; therefore, even further shock wave applications cannot detach the stone fragments.

In principle, every sialolith can be fragmented by means of extracorporeal shock waves, regardless of its mineralogical composition. This was proven by in vitro analyses.[16] In this clinical study as well, signs of fragmentation ensued after every treatment.

Whether, however, the fragmented stone pieces can be detached by means of extracorporeal shock wave lithotripsy and washed out through the secretory duct obviously depends on how firmly the stone is embedded in the secretory duct system, possibly as a consequence of past inflammatory events. Thus, in our opinion, the rate of patients free of stones after treatment depends on the structural environment of the respective parotid stones, rather than on the sialoliths as such. Studies of other authors have not established a proven connection between stone size and the results after extracorporeal shock wave treatment, either. These observations, however, are only valid for a stone size of up to about 12 mm. From that size onward, stone elimination is only possible in special cases.[11]

The assumption that the percentage of patients becoming free of stones is essentially influenced by the relationship between the stone and the structures in its environment would also explain why the success rate of shock wave treatments of stones in the submandibular gland is considerably lower than that of parotid stones.[9-13] The ascending course of the secretory duct and the viscous (seromucous) saliva of the submandibular gland are likely to promote the "impacting" of stones in the secretory duct system. Because of the low risks of the other therapies, the high success rate especially of duct slitting with preservation of the gland, and the altogether disappointing results of the shock wave treatment with free-of-stone rates of mostly under 40%, extracorporeal lithotripsy of stones in the submandibular gland remains reserved for certain indications.[3]

With respect to the significant differences between extracorporeal shock wave treatments of stones of the submandibular gland and of the parotid gland, the results should always be considered separately, although both stones are sialoliths.

While in in vitro studies, the number of shock waves required for total destruction was determined to be dependent on the stone size,[16] our initial clinical experience,[13] in accordance with reports by other authors,[8] did not reveal a connection between stone size, number of shock waves, and the rate of patients being free of stones. Considering this and our experimental results, the number of shock waves we selected per treatment ranged from 2,500 to at most 3,000, not depending on the stone size. Other authors chose between 1,300 and 7,500 shock waves per treatment.[8,10,11]

With a second treatment, the rate of patients becoming free of stones was increased in our group of patients. The third treatment, however, only resulted in a stone-free rate of 10%, so that further shock wave treatments (more than 3 sessions) do not appear expedient. Although as a rule the piezoelectric shock wave application can be carried out without the administration of sedation or analgesia and therefore as an outpatient treatment, we do not suggest more than 3 treatments, and on this point, we agree with other authors.[8]

Patients suffering from symptomatic stone disease of the parotid gland in spite of a threefold shock wave treatment should be directed to submit to surgical therapy in the form of a parotidectomy.

CONCLUSION

Extracorporeal shock wave therapy for treating parotid stones has proven an effective and, also in the long-term course, reliable method of treatment. We consider it — when conservative therapies like gland massage or duct bougienage fail — as the therapy of choice, avoiding in the vast majority of cases a parotidectomy.

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TREATMENT OF CHRONIC SUPPURATIVE OTITIS MEDIA WITH TOPICAL CIPROFLOXACIN

JOSEPH E. DOHAR, MD, MS
PITTSBURGH, PENNSYLVANIA

CUNEYT M. ALPER, MD
PITTSBURGH, PENNSYLVANIA

ELIZABETH A. MCI. ROSE, FRACS
MELBOURNE, AUSTRALIA

WILLIAM J. DOYLE, PHD
PITTSBURGH, PENNSYLVANIA

MARGARETHA L. CASSELBRANT, MD, PHD
PITTSBURGH, PENNSYLVANIA

MARGARET A. KENNA, MD
BOSTON, MASSACHUSETTS

CHARLES D. BLUESTONE, MD
PITTSBURGH, PENNSYLVANIA

To date, only ofloxacin has been approved by the US Food and Drug Administration for treatment of ears with a nonintact tympanic membrane. The purpose of this study was to determine the safety and efficacy of topical ciprofloxacin hydrochloride in the treatment of experimental chronic suppurative otitis media caused by *Pseudomonas aeruginosa* infection in cynomolgus monkeys. Forty adult cynomolgus monkeys were divided into 4 equal groups, and their ears were challenged with *P aeruginosa*, drained for 3 weeks, then treated twice daily for 4 weeks with 1 of 4 randomly assigned agents: 1) ciprofloxacin, 2) saline, 3) Cortisporin, or 4) vehicle. The animals were followed up with auditory brain stem response testing, culture, otoscopy, and histopathology. Both ciprofloxacin and Cortisporin treatment resulted in a significantly more rapid rate of clearance of *P aeruginosa* as compared to treatment with saline (100% versus 20%). Eradication was not associated with resolution of otorrhea after a 4-week period of treatment. There were no significant changes in auditory brain stem response wave latencies for any of the treatment groups. Histopathologic data revealed that there was no statistically significant difference in the amount of outer hair cell loss for the ciprofloxacin group as compared to the control ear and other treatment groups. We conclude, therefore, that topical ciprofloxacin is not ototoxic and is effective in sterilizing the otorrhea, but does not promote resolution of the drainage, in this animal model.

KEY WORDS — chronic suppurative otitis media, ciprofloxacin, otorrhea.

INTRODUCTION

There are several problems with the presently available ototopical preparations for use in ears with a nonintact tympanic membrane. First, there is continued concern over the potential ototoxicity of many of these agents.[1-3] Second, few clinical trials have demonstrated in a prospective, randomized, double-blinded, and placebo-controlled fashion the indications, safety, and efficacy of such agents. Finally, *Pseudomonas aeruginosa*, one of the most common bacteria recovered from otorrhea, has been shown to facilely develop resistance to antibiotics. Consequently, some of the topical antibiotics in current use do not have favorable in vitro susceptibility profiles.[4-6] Clearly, investigation into alternative ototopical agents is necessary.

Ciprofloxacin is a fluorinated quinoline antibiotic, structurally related to nalidixic acid. Because the in vitro susceptibility profile of ciprofloxacin includes the pathogens commonly believed to underlie suppurative otorrhea, there is good reason to study this agent as an ototopical alternative to the currently used agents. The objective of the current study was to determine the safety and efficacy of topical ciprofloxacin in the treatment of experimental chronic suppurative otitis media (CSOM) caused by *P aeruginosa* infection in cynomolgus monkeys.

MATERIALS AND METHODS

Forty adult cynomolgus monkeys of both sexes were randomly assigned to 1 of 4 treatment groups (10 animals per group): saline (negative control group), Cortisporin otic suspension (neomycin, polymyxin B sulfate, and hydrocortisone; Burroughs Wellcome; positive control group), ciprofloxacin 0.2% (experimental group), or vehicle (potassium sorbate, polysorbate 20, sodium acetate trihydrate, glacial acetic acid, glycerin, methocel A4M, and purified water; pH 4.75). All procedures on animals were approved by the Animal Research and Care Committee at Children's Hospital of Pittsburgh (#21/93).

From the Department of Pediatric Otolaryngology, University of Pittsburgh School of Medicine and Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania (Dohar, Alper, Doyle, Casselbrant, Bluestone), the Department of Otolaryngology, Royal Children's Hospital, Melbourne, Australia (Rose), and the Department of Otolaryngology, Boston Children's Hospital, Boston, Massachusetts (Kenna). This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Children's Hospital of Pittsburgh.

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CORRESPONDENCE — Joseph E. Dohar, MD, MS, Dept of Pediatric Otolaryngology, Children's Hospital of Pittsburgh, 3705 Fifth Ave, Pittsburgh, PA 15213.

The sample size chosen was based on the expectation that untreated ears (ie, 100%) would continue to drain culture-positive fluid, as opposed to none of the ears treated with topical antibiotic. A sample size of 10 animals per group allows for the detection of such a difference at $\alpha = 0.05$ and a power of 0.8 (Fisher exact test), the secondary outcome measure (ie, time to resolution of otorrhea). This sample size was sufficient to demonstrate a 50% reduction in the number of draining ears. The animals were quarantined for a 5-week period and then baseline testing consisting of otoscopy (Zeiss OPMI 1 microscope), tympanometry (Teledyne Avionics TA-7A impedance audiometer), and auditory brain stem response (ABR) tests was performed. Under anesthesia (ketamine hydrochloride 20 mg/kg administered intramuscularly), a perforation was made in the right tympanic membrane and confirmed by otoscopy and tympanometry. A postmyringotomy ABR test was performed to document possible threshold shifts resulting from the tympanic membrane perforation. Then the right ears of all animals were challenged with 40 colony-forming units (CFU) of *P aeruginosa* (strain ATCC 27853). One week later, the right ears of the animals numbered 1 through 20 were challenged with 1,000 CFU of *P aeruginosa* (Rochester strain), and the right ears of animals numbered 21 through 40 were challenged with 100,000 CFU of that strain of *P aeruginosa*. Challenges with 100,000 CFU of *P aeruginosa* (Rochester) were repeated 3 days later in all animals (numbers 1 through 40). Otorrhea persisted for 3 weeks following the last challenge. During this period, the ears were examined, cultured (Calgiswab, Fisher Scientific), and suctioned twice per week, but no therapy was given. The ears were evaluated for the presence or absence of tympanic membrane perforation and otorrhea. If drainage was present, the quantity was scored by an otolaryngologist observer on a 5-point scale (0 = none, 1 = moist culture tip, 2 = fluid confined to the middle ear, 3 = fluid observed in the external canal, 4 = fluid observed draining from the external ear canal on surface examination), and the quality of the otorrhea was scored as serous (1), mucoid (2), mucopurulent (3), or purulent (4). The appearances of the tympanic membrane, middle ear mucosa, and external auditory canal were recorded. During each examination, debris was removed from the external ear canal and the drainage was suctioned from the middle ear and external canal. If the tympanic membrane was healed, a new perforation was created under sterile conditions and any existing effusion was cultured.

For each sample, the calcium alginate swab was streaked immediately onto chocolate agar plates (for isolation of any bacteria present) and then onto Pseudocel plates (which select for *P aeruginosa*). All plates were incubated at 37°C and read at 24, 48, and 72 hours. *Pseudomonas aeruginosa* and other bacterial species were identified and their relative abundances recorded at each time point by a microbiologist blinded to group assignment of the animals. Ciprofloxacin sensitivities of randomly selected *P aeruginosa* colonies, as well as colonies of other organisms, were evaluated by standard methods.

At the end of that period, an ABR test was performed in a soundproof booth with the Nicolet Compact Auditory Electrodiagnostic System used for both stimulus generation and potential recordings, and therapy as per group assignment was initiated. All tests were conducted after suctioning of existing fluids from the external canal and middle ear. Two types of stimuli were presented to the test ears: clicks and tone bursts. Tone burst stimuli of 8, 4, and 2 kHz were used. Given that the basal turn of the cochlea is responsible for high-frequency perception and that this is the region in closest proximity to the round window membrane, we chose to use tone bursts to test these specific frequencies. Each stimulus was presented at intensities from 100 dB down to threshold, with thresholds estimated to within 5 dB. For each stimulus, 500 sweeps were recorded, analyzed, and stored. The ABR tests were performed in the monkeys at baseline, repeated after the tympanic membrane was perforated, prior to initiation of therapy (after establishment of CSOM), and then repeated at 2 and 4 weeks during the course of therapy. Ototopical administration of the treatment was performed twice daily for 4 weeks. Biweekly culture and suctioning of the ears was continued over the 4-week period of therapy. The animals were painlessly killed by vital perfusion (rinse solution of cacodylate buffered saline) and fixed with Karnovsky solution in cacodylate buffer, and the temporal bones were harvested and submitted for histopathologic study of the cochlea. Stapedectomy and round window perforation were performed under the otomicroscope, and the perilymph was displaced by fixative solution. The cochleas were placed in fixative for 24 hours and then perfused with and placed into a cacodylate buffer and refrigerated. The temporal bones were then fixed in osmium tetroxide, dehydrated in alcohol, and embedded in plastic agar. For the evaluation of cochlear pathology, the block-surface technique was used as described in the literature.

Surface preparations were examined under the light microscope for the quantification of hair cell loss. Hair cells were counted separately for each position (first, second, and third outer hair cell [OHC] rows) in the first and second parts of the first and

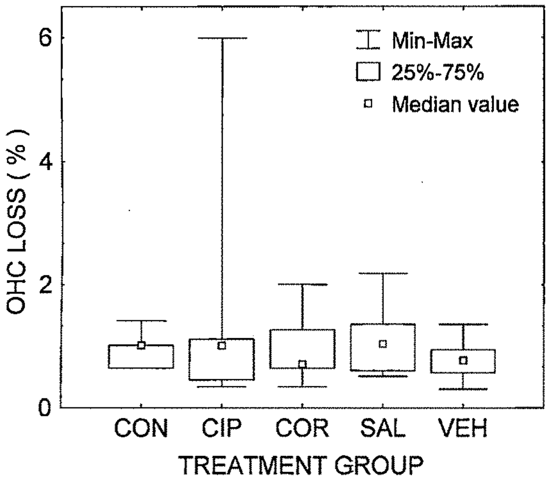

Fig 1. Box and whisker plot of average outer hair cell loss in left, untreated control ears (CON) and right ears treated with ciprofloxacin (CIP), Cortisporin (COR), saline (SAL), or vehicle (VEH).

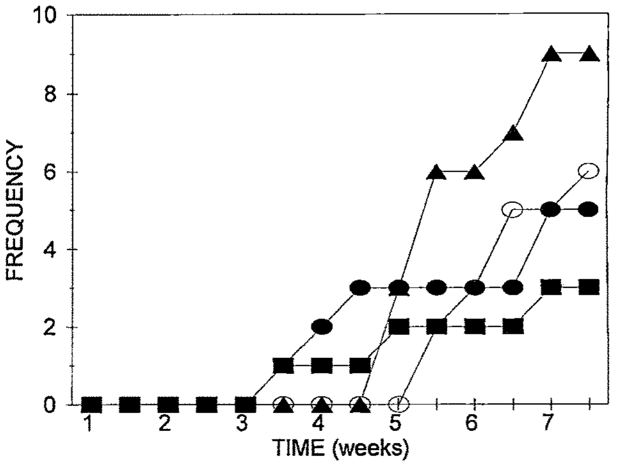

Fig 2. Frequency of tympanic membranes that closed by healing for treatment groups ciprofloxacin (filled circle), vehicle (open circle), Cortisporin (square), and saline (triangle). Note that tympanic membranes in saline group healed more frequently than those in other groups.

second turns (coils) of the cochlea. For each cochlea, results were reported for those 12 counting regions and expressed as a percentage of damaged hair cells.

RESULTS

Safety. Safety was determined by 2 independent means: ABR testing and histopathologic analysis of the cochleas. The ABR parameters specifically considered included latencies and thresholds. As expected for a conductive hearing loss secondary to tympanic membrane perforation, a significant increase was noted after the perforations in the absolute latencies ($p > .05$, paired *t*-test) but not in the I-V interwave latency. No significant latency changes were noted otherwise. Like latency, significant threshold shifts were only observed after subtotal perforation and not otherwise during the study period. A threshold shift was considered significant if it equaled or exceeded 10 dB.

Histopathologic analysis (Fig 1) revealed that there were no significant differences in OHC loss between any of the groups for any combination of locations or rows of OHCs. Specifically, the Figure depicts the OHC loss of the right cochleas in the 4 treatment groups (ciprofloxacin, vehicle, Cortisporin, and saline). A fifth group, labeled in the Figure "CON," represents the data on the OHC loss of the left cochleas of animals without CSOM or treatment intervention in order to provide normative data regarding hair cell loss in this species. The median values for hair cell loss are represented by the small central squares, and the ranges (from maximum to minimum) are represented by the horizontal lines. The larger rectangle represents the OHC counts 25% and 75% above the minimum value. Pearson correlation coefficients revealed no correlation between average OHC loss and hearing loss when overall regions and rows were compared to tested frequencies. It should be noted that none of our samples had strial changes or inner hair cell loss.

To investigate the correlations between the hearing results and OHC loss, the data for OHC loss averaged by location and position were compared with the hearing loss at each examined frequency and also with the average hearing loss. Pearson correlation coefficients between the hearing results and histopathologic results were computed. There were no significant correlations between average OHC losses in the various regions or rows and the hearing losses at the tested frequencies. There were a few significant correlations when the analysis was made between specific frequencies and hair cell location and/or rows, but these most likely reflect the consequences of multiple comparisons. This relationship was also explored by evaluating the animals that had significant hair cell losses or hearing losses on a case-by-case basis. It is interesting to note that there were no concurrent hearing losses in the animals with a relatively large OHC loss (except within the range of the mild category). Conversely, there were no abnormal OHC losses in the few monkeys that had up to a 20-dB hearing loss at certain frequencies and up to a 7.5-dB average hearing loss.

Efficacy. Five outcome measures were used to determine treatment efficacy: closure of the tympanic membrane perforation, resolution of otorrhea, quantity of otorrhea, quality of otorrhea, and eradication of *P aeruginosa*.

Figure 2 shows the cumulative frequency distribution of the time to first observation of a healed tympanic membrane. Overall, the tympanic mem-

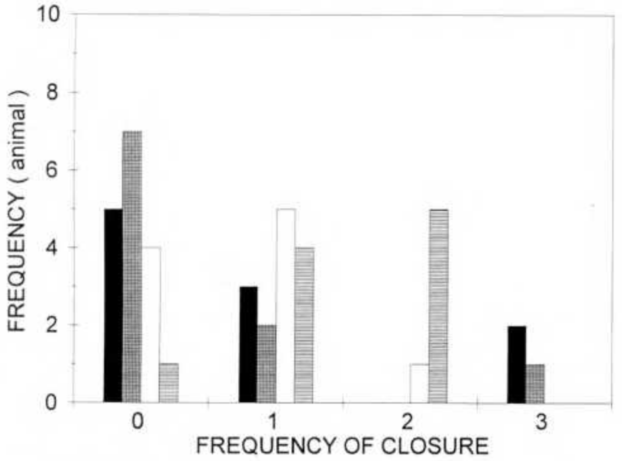

Fig 3. Bar graph demonstrating frequency of tympanic membrane closure for animals in treatment groups ciprofloxacin (black), vehicle (white), Cortisporin (squares), and saline (horizontal lines).

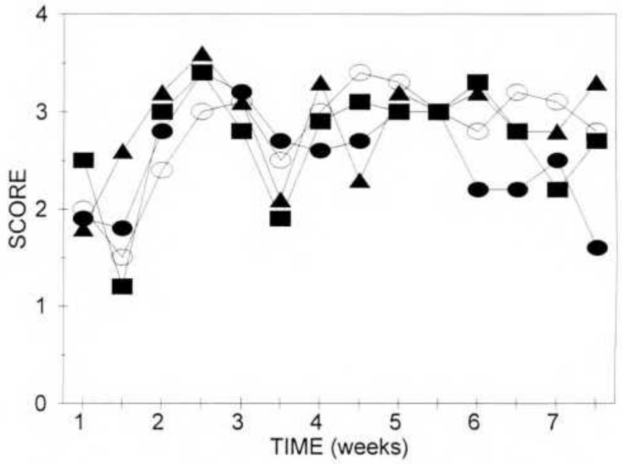

Fig 4. Average drainage scores during study period for treatment groups ciprofloxacin (filled circle), vehicle (empty circle), Cortisporin (square), and saline (triangle).

branes of 9 ears in the saline-treated group, 6 in the vehicle-treated group, 5 in the ciprofloxacin-treated group, and 3 in the Cortisporin-treated group healed spontaneously during the period of follow-up. A new myringotomy was made in these ears, and in the majority of cases, the continued presence of an effusion within the middle ear space was documented. Figure 3 is a bar graph displaying the number of ears in the 4 groups whose tympanic membranes had not healed during the follow-up, and those that had healed 1, 2, and 3 times.

There were no differences between groups in the ratings of otorrhea quantity or quality over the period of follow-up. Of the 8 otomicroscopic examinations made during the period of treatment, an average of 1.4 ± 1.1 were rated 0 (otorrhea-free) in the ciprofloxacin group, as compared to 0.2 ± 0.4 observations in the Cortisporin group, 1.1 ± 1.1 in the saline group, and 0.4 ± 0.8 in the vehicle treatment group. Figure 4 shows the average score for quantity of otorrhea (0 to 4) for the 4 treatment groups as a function of time. For all groups, average scores of between 2 and 3 were observed throughout the period of study, with no significant differences documented between groups.

By 3 weeks of treatment, all ears in the ciprofloxacin and Cortisporin groups were negative for *Pseudomonas*. In contrast, 8 of the ears in the saline treatment group were still culture-positive for *Pseudomonas* at the end of the study. Of interest, the vehicle resulted in an eradication rate intermediate between the rates of the saline and ciprofloxacin treatment groups (Fig 5). Kaplan-Meier survival analysis of these data showed the functions for the ciprofloxacin ($p < .05$) and Cortisporin ($p < .05$) groups to be significantly different from that of the saline group, but not the vehicle group ($p > .15$). The difference in eradication rates for the vehicle and saline groups was not statistically significant ($p > .15$).

DISCUSSION

Ciprofloxacin has a broad spectrum of activity against both gram-positive and gram-negative bacteria, and greater potency than the cephalosporins and aminoglycosides. It has excellent potency against Enterobacteriaceae: fastidious gram-negative bacilli such as *Haemophilus influenzae*, *Neisseria meningitides*, *Neisseria gonorrhea*, and *Moraxella catarrhalis*. This includes activity against β-lactamase–producing organisms. Ciprofloxacin is active against *Pseudomonas*, especially *P aeruginosa*, and has greater activity than other antipseudomonal agents. It has good activity against *Staphylococcus*, including *Staphylococcus aureus*, but is less active against *Streptococcus* and *Enterococcus*. There is minimal

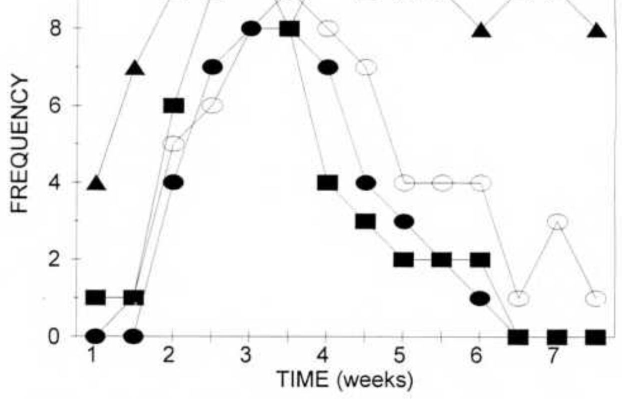

Fig 5. Frequency during study period of ears culture-positive for *Pseudomonas aeruginosa* for treatment groups ciprofloxacin (filled circle), vehicle (empty circle), Cortisporin (square), and saline (triangle).

activity against anaerobes and none against *Candida albicans*. There is also activity against *Mycobacterium, Rickettsia, Coxiella burnetii*, and *Plasmodium falciparum*.[7,8]

Ciprofloxacin has been used in human studies, both systemically and topically. In diffuse external otitis there was a 100% cure rate with both 0.2% and 0.5% solutions of ciprofloxacin. For ears with CSOM there was an 80% cure for both drop strengths[9]; in other studies there was an 85% cure rate after topical administration of ciprofloxacin drops to chronically discharging ears[10] and a 95% cure when ciprofloxacin was used orally.[11]

Fluoroquinolone antibiotics are generally well tolerated and are rarely associated with serious or life-threatening adverse reactions.[7] Studies have found the major adverse reaction to systemic ciprofloxacin to be arthropathy in the weight-bearing synovial joints of immature animals,[12,13] which was manifested clinically as a limp due to swelling of the joint. On histopathologic examination there were changes in the cartilage with blister formation and joint erosion, and chondrocyte necrosis followed by dissolution of the matrix throughout the articular cartilage. These degenerative changes were irreversible.[14] There is a species difference found: in dogs, 25% of the animals given a therapeutic dose developed arthropathy; in rats given 100 times the therapeutic dose, only 5% developed arthropathy.[15] Oral ciprofloxacin has now been given, on compassionate grounds, to children with cystic fibrosis as treatment for their *Pseudomonas* lung infections, and in reports of more than 1,000 children there has been no evidence of clinical or radiologic arthropathy.[15,16] There have been reports of arthralgia in older girls with cystic fibrosis (13 years and older) that reverses when the drug is discontinued.[16] This may be due to circulating immune complexes from their underlying pulmonary and gastrointestinal disease[17,18] rather than to an effect of the ciprofloxacin.

The potential for ototoxicity due to the topical administration of antibiotics in the setting of a nonintact eardrum has been a concern for several years. Toxicity to the middle and inner ear has been demonstrated in several animal studies.[19-27] The aminoglycosides reach high levels in perilymph regardless of whether the administration is topical or systemic, and corresponding sensory hair cell loss has been found.[24] Although theoretic concern exists over the possibility of hearing loss in humans due to these agents, only anecdotes have been published to support this concern.[28] In spite of this dearth of documentation in humans, the toxicologic findings in animals have precluded a Food and Drug Administration indication for the use of currently marketed antibacterial ototopical preparations when the tympanic membrane is not intact. This has provided some of the impetus to continue the search for alternative agents.

Topical ciprofloxacin in guinea pigs has been shown to result in hearing loss of less than 10 dB at 4 kHz and 8 kHz that is thought, but not proven histologically, to be due to mucosal changes.[17] No hair cell loss was observed in the organ of Corti in these animals. An additional study[29] revealed that 1/600th of the ciprofloxacin administered to the middle ear passed through the round window membrane and was recovered from the perilymph. This did not result in an alteration of hair cell morphology. Ciprofloxacin (0.2%) and ciprofloxacin (0.2%) with hydrocortisone were shown to be relatively safe when used topically for the treatment of subacute suppurative otorrhea in guinea pigs.

Our data in this study further support the conclusion from the earlier studies[17,29] that topical ciprofloxacin (0.2%) is relatively safe for the treatment of CSOM. This was true for functional analysis of hearing using ABR testing, as well as for histopathologic analysis of the inner ear using ultrastructural and surface techniques. Although no significant group differences were documented demonstrating threshold shifts of at least 10 dB, we did not expect them, due to the low power of a small sample size in each group (n = 10). It was curious that 2 ears in the ciprofloxacin group experienced significant threshold shifts at 2 frequencies, but a similar result was obtained in the saline control group. The biologic significance of such a finding is confounded by the variability in hearing thresholds recorded over time in all treatment groups.

It was hoped that the histopathologic data might help to resolve this dilemma in the functional results. Overall, we found that there was no statistically significant difference in the amount of OHC loss for the treatment groups as compared to the control ear (the left ear, which was not manipulated). This was true overall, as well as for specific loci within the cochlea. Approximately 4% to 5% of OHCs in the basal turn and 10% to 15% in the apical turn may be missing in normal specimens. Further, there is not a perfect correlation between OHC loss and hearing loss. In fact, substantial cochlear damage must occur before there is a definite ABR threshold shift. These issues complicate any attempt to correlate histopathology of the temporal bone with functional ABR examination. We believe that it is reasonable to conclude from our data that since substantial damage was not seen in the animals with the largest threshold shifts, significant ototoxicity did not result from any of the treatment alternatives. Curiously, this was even true for Cortisporin, further supporting either interspecies variation or protection afforded to the inner ear due to thickening and subsequent decreased permeability of the round window membrane with infection.

There were 2 other observations made during our efficacy analysis that warrant discussion. First, a greater number of tympanic membranes healed in the saline group than in any other group. Although this raises the possibility that the other 3 preparations in some way retarded the wound healing, the sample sizes were too small to draw such a conclusion. Further, no tissue analysis was done, since tissue interaction with the various topical preparations was not an objective of this study. Alternatively, the seemingly enhanced capacity of the tympanic membrane treated with saline to heal may have been a result of persisting infection and inflammation of the tympanic membrane in that group. Although not specifically demonstrated, one would expect that in the group treated only with saline, the infection and resulting inflammation would persist longer than in the groups treated with a topical antibiotic. As noted, the majority of these ears with healed tympanic membranes had effusions at the time a new myringotomy was made. Several investigators have reported that bacterial infection and resulting inflammation accelerate wound healing.[30-33] It is plausible, therefore, that our finding is explained by this mechanism. Further research is necessary to fully understand this observation.

Second, it was interesting that eradication of the infecting organism did not correlate with cessation of otorrhea. We speculated that our follow-up may not have been long enough to detect a difference in this outcome measure. Another possible explanation is that although the organism was eradicated, the mucosal inflammation may not have been entirely treated. Of our experimental groups, the only one that received an anti-inflammatory agent was the Cortisporin group. Although a steroid, hydrocortisone, is present, it is a relatively weak topical steroid. Although little attention has been paid by otolaryngologists to the topical steroid component of ear drops, vast differences between various topical steroids in eyedrops have been found,[34] and this is one area that needs considerably more attention as new ototopical preparations are developed.

CONCLUSION

1. Chronic suppurative otitis media secondary to *Pseudomonas* infections in monkeys does not cause significant conductive or sensorineural hearing loss.

2. Ototopical treatment of CSOM caused by *P aeruginosa* with ciprofloxacin, Cortisporin, or the vehicle for Cortisporin in monkeys is not ototoxic.

3. Ototopical Cortisporin and ciprofloxacin eradicated *P aeruginosa* more rapidly from the otorrhea as compared to saline, but this was not correlated with resolution of the inflammation after 4 weeks of treatment.

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BILATERAL SIMULTANEOUS HEARING PRESERVATION MASTOIDECTOMY IN OTOGENIC MENINGITIS

ANAND JOB, MS
ANNIE JACOB, MS
MARY KURIEN, MS
JOHN MATHEW, MS

VELLORE, INDIA

Bilateral simultaneous hearing conservation mastoidectomy has not been previously documented. We present our experience with this procedure. Three patients with pyogenic meningitis secondary to bilateral unsafe ears had hearing preservation surgeries of both ears in a single sitting. Although ear surgery carries risk of inner ear damage, in our series there was no deterioration of inner ear function in the 6 ears operated on. The air conduction threshold improved in 3 (50%) of the ears. When meningitis results from bilateral otogenic foci as in our series, simultaneous mastoidectomy in both ears may be the preferred method of treatment to eradicate the source of infection.

KEY WORDS — bilateral simultaneous hearing conservation mastoidectomy, bilateral unsafe ears, otogenic meningitis.

INTRODUCTION

Bilateral simultaneous mastoidectomy has been contraindicated for fear of producing bilateral dead ears. In pyogenic meningitis resulting from an ear in which there is a life-threatening situation, the source of infection needs to be dealt with, and mastoidectomy is done as a part of the management.[1,2]

When both ears are unsafe, the ear that produced the complication is dealt with first, followed by appropriate treatment for the other ear at a later date, to reduce the risks of sensorineural hearing loss.[3,4] It is known that active unsafe ear disease can itself lead to sensorineural hearing loss of various degrees and even a dead ear,[5] and that a dry ear can be considered a safe ear.[6]

It is well documented that intracranial complications secondary to chronic suppurative otitis media can occur from extension through bony defects due to resorption by osteitis or cholesteatoma, or through an infected clot that passes within a small vein through bone and dura, where apparently intact bone may be transgressed by thrombophlebitis within its haversian vascular system or through normal anatomic pathways.[1,2] It is also accepted that radiologic investigation in chronic mastoiditis is of little value, as chronic infection has already caused periosteal irritation and hyperostosis in which the density of the bone makes the detection of an underlying lytic area difficult unless the bone destruction is extensive.[2] A computed tomography scan is done in otogenic meningitis, primarily to rule out other coexisting intracranial complications.[1,2,7] In a case series reported by Kangsanarak et al,[7] 36% of cases were found to have other associated intracranial complications.

Intracranial complications have been reported in both cholesteatomatous (58%)[7,8] and noncholesteatomatous (21%[7] or 42%[8]) chronic suppurative otitis media. The preferred treatment for the focus of infection in otogenic intracranial complications is radical mastoidectomy[1,2,7,8] or modified radical mastoidectomy in cholesteatomatous ears and cortical mastoidectomy[8] in noncholesteatomatous ears.

Unsafe chronic suppurative otitis media with meningitis, with or without cholesteatoma, with or without bone destruction, requires immediate local care to the infected ear and a mastoidectomy as soon as the meningitis is controlled with appropriate parenteral antibiotics and the patient can withstand surgery; the type of surgery depends upon the operative findings.[2,7,8]

When meningitis occurs as a complication of bilaterally unsafe ears, with no other localizing clinical or radiologic features, it is difficult to decide which the offending ear is, unless the middle ear and mastoid are explored under general anesthesia. In such situations, we decided to perform bilateral mastoidectomy in one sitting to eradicate the focus of infection and to preserve useful hearing. Hearing preservation procedures were done in all our patients, despite the fact that a radical procedure had been previously performed.[1] Hence, we report our experience in this difficult clinical situation.

CASE REPORTS

The 3 patients in this series were admitted to the

From the Department of ENT, Christian Medical College and Hospital, Vellore, India.

CORRESPONDENCE — Mary Kurien, MS, Dept of ENT, Christian Medical College and Hospital, Vellore 632 004, Tamil Nadu, India.

TABLE 1. CLINICAL PROFILE

	Patient 1	*Patient 2*	*Patient 3*
Age (y)	11	26	24
Sex	Male	Male	Male
Bilateral ear discharge (y)	8	15	10
Otoscopy	Bilateral attic cholesteatoma	Right PSRP with pus, left PSRP with cholesteatoma	Right PSRP with cholesteatoma, left posterior marginal perforation with pus
CT scan of brain	Normal	Normal	Normal
Ear swab culture	*Enterococcus faecalis, Citrobacter diversus*	Coagulase-negative staphylococci	*Pseudomonas*
Medical treatment	Parenteral CP + Chl for 14 days, followed by oral sulfa for 14 days	Parenteral CP + Chl for 14 days, followed by oral sulfa for 14 days	Parenteral ampicillin + ciprofloxacin for 14 days, followed by oral sulfa for 14 days

PSRP — posterosuperior retraction pocket, CT — computed tomography, CP — crystalline penicillin, Chl — Chloromycetin, sulfa — sulfamethoxazole + trimethoprim.

Christian Medical College and Hospital, Vellore, which is a tertiary referral center in South India, between 1993 and 1995 with the diagnosis of meningitis secondary to an otogenic cause. They were all found to have bilateral unsafe ears on the basis of the history and otoscopic findings (attic cholesteatoma, marginal perforation, posterior superior retraction pockets with active purulent discharge)[1,2] associated with moderate to severe hearing loss. The clinical profile, treatment, operative findings, and outcome are given in Tables 1 and 2. Hearing thresholds were calculated for each ear air conduction pure tone average for 0.5, 1, and 2 kHz, as were bone conduction averages and air-bone gap averages in the same frequencies. All patients were given parenteral antibiotics with aural toilet for more than a week prior to mastoid exploration (Table 1). The operative procedure done depended on the preoperative audiogram and the perioperative findings (Table 2). Cholesteatoma was seen in 4 of the 6 ears, and granulation tissue in 2 of the 6 ears. Bony defects were identified in only 2 of the ears. In 1 patient no bony defect could be identified in either ear (Table 2). There were no significant intraoperative or postoperative complications in any of these patients. In all of our patients, we were able to avoid radical mastoid surgery, and either modified radical mastoidectomy or atticotomy along with cortical mastoidectomy was done (Table 2). The postoperative audiograms in all cases showed a conductive loss with useful hearing still present, which is to be reconstructed at a second stage (Figs 1 and 2). A follow-up performed 6 to 8 months postoperatively showed that all 3 patients had dry ears and there was no deterioration of hearing.

DISCUSSION

In chronic suppurative otitis media with intracrani-

TABLE 2. OPERATIVE DETAILS

	Patient 1	*Patient 2*	*Patient 3*
Operative procedure done (day of antimeningitic therapy)	Bilateral MRM (10th day)	Right CM + AT, left MRM (14th day)	Right MRM, left CM + AT (10th day)
Operative findings			
Attic and antrum			
Cholesteatoma	Bilateral	Left	Right
Granulations	Left	Right	Left
Bony defects	Left (tegmen and sinus plates)	None	Right (tegmen)
Ossicles			
Malleus	Bilateral	Right	Left
Incus	Left*	Right	None†
Stapes (superstructure)	Left (embedded in granulation)	Bilateral	Left
Duration of surgery	6 h	5 h	5 h
Duration of hospital stay	18 d	26 d	14 d

MRM — modified radical mastoidectomy, CM — cortical mastoidectomy, AT — atticotomy.

*Lenticular process of incus had necrosis in right ear.

†Lenticular process of incus had necrosis in left ear.

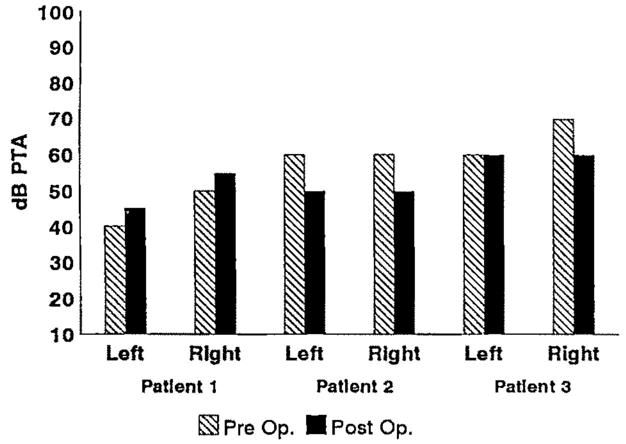

Fig 1. Air conduction pure tone average (PTA) threshold values.

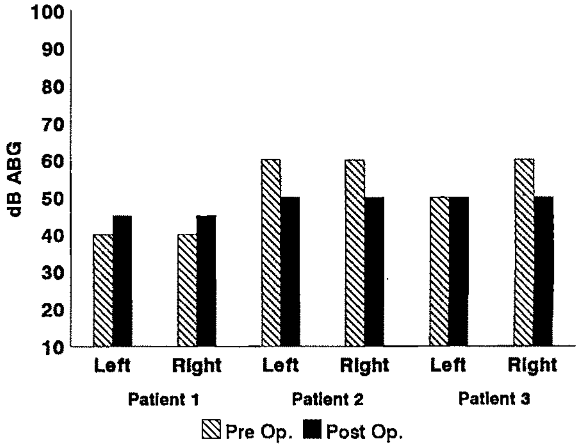

Fig 2. Air-bone gap (ABG).

al complications, the primary concern is to treat the intracranial disease and then to prevent reinfection by treating the ear.[1,2] In otogenic meningitis, as coexisting intracranial complications have been reported to occur in 36% of cases,[7] it is mandatory to do computed tomography or magnetic resonance imaging.[1,2,7] Following the treatment of complications, mastoidectomy is done as early as possible to remove the source of infection, to prevent further intracranial complications, to arrest the progress of the ear disease, and to preserve useful hearing.[1,2,7-9]

In meningitis with no other associated localizing intracranial complication, and when both ears are unsafe, it is difficult to decide which the culprit ear is, even with radiologic investigations. In such a life-threatening situation, it is ideal to eradicate the sources of infection completely as early as possible. When bilateral surgery is done for extensive disease of the ear and mastoid, the possibility of injury to the facial nerve and inner ear[3,4] is an added risk. However, because of the morbidity and mortality of meningitis and the possibility of reinfection that can occur subsequently if the focus of infection is still present, bilateral simultaneous mastoidectomy was considered the treatment of choice in our cases. Bony defects were seen in only 2 of the ears, and 1 patient had no detectable bony defects. The possible means of intracranial spread can be through routes other than bony defects.[1,2] Cholesteatoma was seen in only 67% (4/6) of the ears operated on in our series, which is comparable to previous reports of 58%.[7,8] Though modified radical mastoidectomy was done in the cholesteatomatous ears and atticotomy with cortical mastoidectomy was done in the noncholesteatomatous ears, there was no evidence of recurrence of intracranial complications, and no dead ears, in this series. In fact, there was significant improvement of middle ear function in more than half of the ears (Figs 1 and 2). This outcome shows that in spite of intracranial complications in unsafe ears, a dry, safe ear with useful hearing could be attained with a less radical procedure.

CONCLUSION

Intracranial complications can occur in attic, posterosuperior disease, or marginal perforation with cholesteatoma or granulations, in both cholesteatomatous and noncholesteatomatous ears with or without bony defects. In otogenic intracranial complications, although the principal aim is to treat the life-threatening complications, it is mandatory to eradicate the source of infection as early as possible, thereby producing an ear that is dry and safe with useful hearing. Therefore, in cases of meningitis occurring secondary to bilateral unsafe chronic suppurative otitis media, with no other localizing intracranial complications, bilateral simultaneous hearing preservation mastoidectomy may be considered a relatively safe procedure in a tertiary referral center.

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ADVANCED EAR SURGERY

A Compact Training Course in Advanced Ear Surgery (Endaural Tympano-meatoplasty and Enlarged Middle Fossa Approach) will be held May 16-19, 1999, in Erlangen-Nuremberg, Germany. For information, contact Prof Dr M. E. Wigand, Dept of ORL, University of Erlangen-Nuremberg, HNO Universitatsklinik, Waldstrasse 1, D-91054, Erlangen, Germany; telephone +49/9131/85-3141; fax +49/9131/85-6857.

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EFFECTS OF PLATELET ACTIVATING FACTOR ON THE TUBOTYMPANIC MUCOSA AND INNER EAR IN THE GUINEA PIG

YOSHIHARU OHNO, MD
YOSHIHIRO OHASHI, MD YOSHIKAZU SUGIURA, MD
HIDEKI OKAMOTO, MD YOSHIAKI NAKAI, MD
OSAKA, JAPAN

The effect of platelet activating factor (PAF) was studied to elucidate its role in the pathogenesis of otitis media and sensorineural hearing loss. The PAF alone did not induce a reduction of ciliary activity of the cultured middle ear mucosa. However, a dose-dependent decrease in ciliary activity was observed in the presence of the medium containing both PAF and macrophages. Intravenous injection of PAF did not induce dysfunction of the mucociliary system or morphologic changes of epithelium in the tubotympanum, but cytoplasmic vacuolization and ballooning were observed in the inner ear within 1 hour after injection of PAF. In contrast, intratympanic injection of PAF induced mucociliary dysfunction and some pathologic changes in the tubotympanum. Intratympanic inoculation of PAF induced no pathologic findings in the inner ear. These results suggest that PAF is at least partially involved in the pathogenesis of certain middle ear diseases such as otitis media with effusion. Additionally, PAF might be involved in the pathogenesis of some types of unexplained sensorineural hearing loss.

KEY WORDS — inner ear, middle ear, mucociliary function, platelet activating factor.

INTRODUCTION

Platelet activating factor (PAF), a relatively recently discovered mediator, was described originally as a product of antigen-induced degranulation of rabbit basophils that is able to stimulate platelet aggregation.[1] This potent mediator has now been found to be generated from precursor ether-linked phospholipids by several cell types, including macrophages, neutrophils, eosinophils, and endothelial cells, following a variety of physiologic and nonphysiologic stimuli.[2-4] This mediator is involved in inflammatory reactions and allergic reactions of a variety of organs through a wide range of biologic activities, including platelet aggregation, increased vascular permeability, recruitment as well as activation of inflammatory cells, and bronchoconstriction.[5-7]

Otitis media with effusion (OME) is a type of inflammatory as well as allergic disorder of the tubotympanum, and an increased vascular permeability is considered to be the origin of middle ear effusions (MEEs).[8] Therefore, PAF might play an important role in the pathogenesis of OME.

Receptors for PAF have been detected in sensory organs and nervous systems.[9] In addition, PAF is synthesized in sensory organs and is able to modulate neurotransmitter release.[10] These data tempt us to conclude that PAF could affect sensory organs, including the inner ear, and might therefore be involved in some types of inner ear disorders. However, few studies have documented the effect of PAF on the tubotympanum or the inner ear. Our study was designed to experimentally elucidate the effect of PAF on the middle ear and inner ear.

MATERIALS AND METHODS

EFFECT ON CILIARY ACTIVITY OF IN VITRO MIDDLE EAR MUCOSA

Seventy-five female Hartley strain guinea pigs (specific pathogen–free [SPF]; mean body weight 200 g) were used. Two mucosal samples (mean thickness 30 μm) were obtained from the middle ear mucosa near the bilateral tympanic orifices of each animal. Each mucosal sample was placed in a sealed chamber containing 3 mL of RPMI 1640 medium solution.[11,12] Thus, a total of 150 mucosal cultures were prepared. First, about 30 minutes after preparation, the ciliary activity (beats per minute) of the most active ciliated cell of each mucosal sample was determined in the presence of RPMI 1640 medium at an ambient temperature of 30°C, by the use of our photoelectric method.[13] Next, each medium solution was replaced with 1 of 15 kinds of solution (labeled A through O; see Table). Every 10 cultures were used to examine the effect of each solution on ciliary activity. The ciliary activity of the cell, whose baseline activity was predetermined, was measured according to the same photoelectric method at the following intervals: 5, 10, 15, 20, 30, and 40 minutes after the solution replacement. In this study, ciliary activ-

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From the Department of Otolaryngology, Osaka City University Medical School, Osaka, Japan.

CORRESPONDENCE — Yoshihiro Ohashi, MD, Dept of Otolaryngology, Osaka City University Medical School, 1-5-7 Asahimachi, Abeno, Osaka 545, Japan.

SOLUTIONS USED TO TEST CILIARY ACTIVITY

Solution	*Contents*	*N*
A	Physiologic saline	10
B	BALF	10
C	Supernatant fluid of BALF	10
D	PAF (10^{-10} mol/L)	10
E	PAF (10^{-9} mol/L)	10
F	PAF (10^{-8} mol/L)	10
G	PAF (10^{-10} mol/L) + BALF	10
H	PAF (10^{-9} mol/L) + BALF	10
I	PAF (10^{-8} mol/L) + BALF	10
J	PAF (10^{-10} mol/L) + supernatant fluid of BALF	10
K	PAF (10^{-9} mol/L) + supernatant fluid of BALF	10
L	PAF (10^{-8} mol/L) + supernatant fluid of BALF	10
M	PAF (10^{-10} mol/L) + whole blood	10
N	PAF (10^{-9} mol/L) + whole blood	10
O	PAF (10^{-8} mol/L) + whole blood	10

Solution B was adjusted to contain 2.0×10^6 cells per milliliter. Solution C was obtained by centrifugation of bronchoalveolar lavage fluid (BALF; 3,000 rpm, 10 minutes). Solutions D, E, and F were 2% fetal bovine serum solution containing 10^{-10}, 10^{-9}, and 10^{-8} mol/L platelet activating factor (PAF), respectively. Solutions G, H, and I were 2% fetal bovine serum solution containing BALF and 10^{-10}, 10^{-9}, and 10^{-8} mol/L PAF, respectively. These were adjusted to contain 1.8×10^6 cells per milliliter. Solutions J, K, and L were 2% fetal bovine serum solution containing supernatant fluid of BALF and 10^{-10}, 10^{-9}, and 10^{-8} mol/L PAF, respectively. Solutions M, N, and O were 2% fetal bovine serum solution containing 10^{-10}, 10^{-9}, and 10^{-8} mol/L PAF and heparinized whole blood of animals from which mucosal samples were obtained. These were adjusted to contain 3.0×10^8 cells per milliliter.

ity was determined 5 times, and the average values were used for analysis. The ambient temperature was kept at 30°C during the experiment by an incubator. The data obtained were statistically analyzed by Wilcoxon's signed-rank test, and significance was accepted when $p < .01$.

EFFECT OF INTRAVENOUS INJECTION

One hundred forty female Hartley strain guinea pigs (SPF; body weight 200 g) were used. They were divided into 2 groups (saline group and PAF group). The 70 animals of the saline group were treated with intravenous injection of 0.1 mL of physiologic saline solution. The 70 animals of the PAF group were treated with intravenous injection of 0.1 mL of 100 ng/mL (2×10^{-7} mol/L) PAF solution. Groups of 10 animals from the saline group and the PAF group were used for the following examinations at 10 minutes, 30 minutes, 1 hour, 3 hours, 1 day, 3 days, and 7 days postinjection.

Otoscopic Examination. The possible presence of MEEs was estimated by the use of a surgical microscope.

Ciliary Activity of Tubotympanum. The right ears of 5 animals from each of the 2 groups were used for the examination of ciliary activity at 10 minutes, 30 minutes, 1 hour, 3 hours, 1 day, 3 days, and 7 days postinjection. Ciliary activity from the 3 different locations within the tubotympanum (midportion of the eustachian tube, middle ear close to the tympanic orifice, and middle ear far from the tympanic orifice; Fig 1) was determined in the presence of RPMI 1640 medium at an ambient temperature of 30°C, by the use of our photoelectric method.[13] Ciliary activity was determined 5 times, and the average values were used for analysis. During the determination, the ambient temperature was kept at 30°C by an incubator. The data obtained were statistically analyzed by a Mann-Whitney U-test, and significance was accepted when $p < .01$.

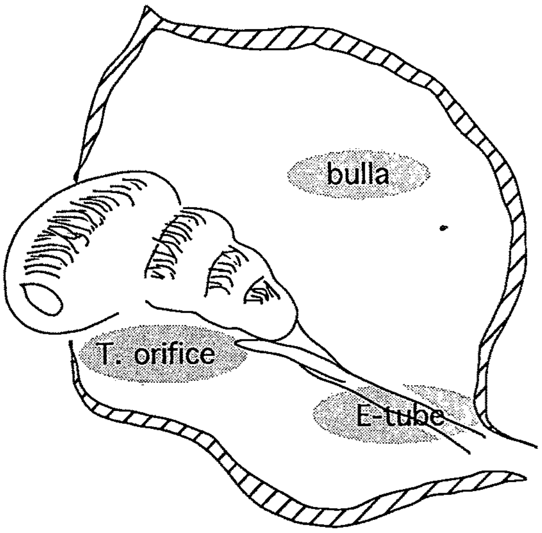

Fig 1. Three locations in tubotympanum where ciliary activity was measured. E-tube — midportion of eustachian tube, T. orifice — middle ear close to tympanic orifice, bulla — middle ear far from tympanic orifice.

Mucociliary Clearance of Tubotympanum. Five animals (10 ears) from each of the 2 groups were used for the examination of mucociliary clearance time of the tubotympanum (MCTT) at 10 minutes, 30 minutes, 1 hour, 3 hours, 1 day, 3 days, and 7 days postinjection. The MCTT was determined by the use of our dye transport technique.[14] In brief, MEE was aspirated under general anesthesia if present. The palate was split to allow visualization of the pharyngeal orifice. Then, 0.15 mL of indigocarmine dye solution was slowly injected into the tympanic cavity through the tympanic membrane. The interval (seconds) required for the first appearance of the dye at the pharyngeal orifice was timed. The Mann-Whitney U-test was used for statistical analysis, and significance was accepted when $p < .01$.

Morphology of Tubotympanum and Inner Ear. The left ears of 5 animals from each of the 2 groups were

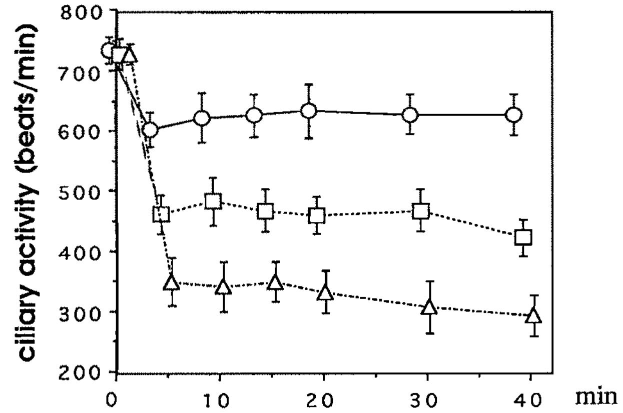

Fig 2. Ciliary activity before and after replacement of solutions G through I. Ciliary activity is significantly reduced in presence of solution G (circles; n = 10 cultures; 10^{-10} mol/L platelet activating factor [PAF]), solution H (squares; n = 10 cultures; 10^{-9} mol/L PAF), and solution I (triangles; n = 10 cultures; 10^{-8} mol/L PAF) in dose-dependent fashion.

used for the examination of the morphology of the tubotympanum and inner ear at 10 minutes, 30 minutes, 1 hour, 3 hours, 1 day, 3 days, and 7 days postinjection. The morphology of the tubotympanum and the inner ear was examined under light and electron microscopes. The animals were painlessly sacrificed by decapitation under general anesthesia. Samples for electron microscopy were immediately immersed in 2.5% cold glutaraldehyde in 0.1 mol/L phosphate buffer solution and fixed for 2 hours. They were transferred to buffer solution for washing and postfixed for 2 hours by 2% osmium tetroxide in the same buffer. After dehydration, samples for scanning electron microscopy were critical point dried, coated with gold, and then observed under an S-405 scanning electron microscope (Hitachi, Japan). Samples for transmission electron microscopy were embedded in Epon 812 after dehydration. Then, ultrathin sections were made with an ultramicrotome (Sovall MT2B) and examined under an H-300 transmission electron microscope (Hitachi) at an accelerating voltage of 100 kV.

EFFECT OF INTRATYMPANIC INJECTION

Sixty female Hartley strain guinea pigs (SPF; body weight 200 g) were used. They were divided into 2 groups (saline group and PAF group). The 30 animals of the saline group were treated with intratympanic injection of 0.1 mL of physiologic saline solution, and the 30 animals of the PAF group were treated with intratympanic injection of 0.1 mL of 100 ng/mL (2×10^{-7} mol/L) PAF solution. Then, 10 animals from each of the 2 groups were painlessly sacrificed at 3, 6, and 14 days postinjection. At the time of sacrifice, they were examined for possible presence of MEEs, ciliary activity, mucociliary clearance, and morphology with light and electron microscopes, according to the methods described above.

In brief, the right ears of 5 animals from each of the 2 groups were used for the examination of ciliary activity at 3, 6, and 14 days postinjection. Ciliary activity from the 3 different locations within the tubotympanum was determined in the presence of RPMI 1640 medium at an ambient temperature of 30°C, by the use of our photoelectric method.[13] The data obtained were statistically analyzed by the Mann-Whitney U-test, and significance was accepted when $p < .01$. Five animals (10 ears) from each of the 2 groups were used for the examination of the MCTT at 3, 6, and 14 days postinjection. The MCTT was determined by the use of our dye transport technique.[14] The left ears of 5 animals from each of the 2 groups were used for the examination of the mor-

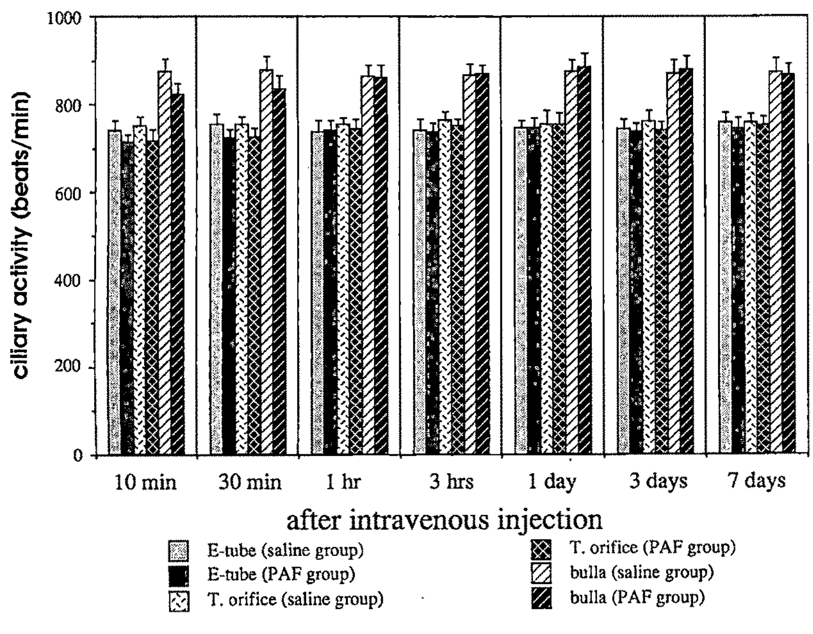

Fig 3. Ciliary activity following intravenous injection of PAF. Ciliary activity is not significantly changed throughout observation period.

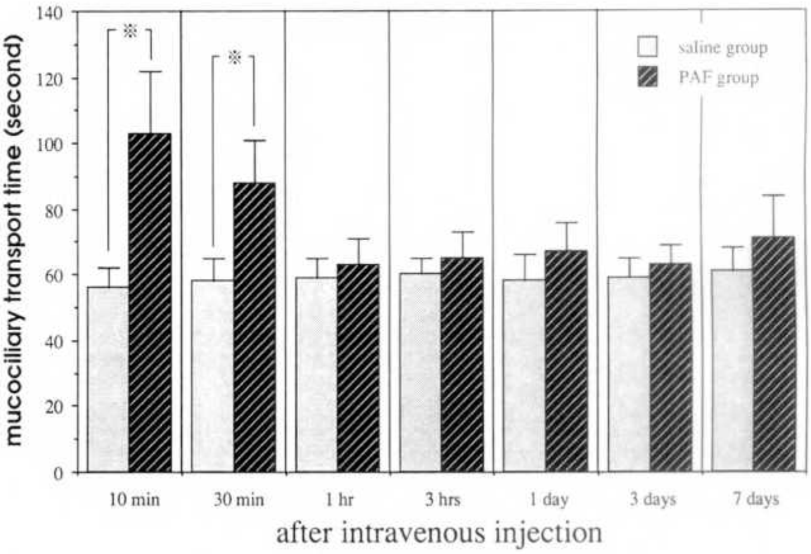

Fig 4. Mucociliary clearance time of tubotympanum (MCTT) following intravenous injection of PAF. MCTT of PAF group at 10 minutes and 30 minutes is significantly longer (※) than that of saline group. However, MCTT of PAF group at 1 hour is not significantly different from that of saline group.

phology of the tubotympanum and inner ear at 3, 6, and 14 days postinjection. The morphology of the tubotympanum and inner ear was examined under light and electron microscopes.

RESULTS

EFFECT ON CILIARY ACTIVITY OF IN VITRO MIDDLE EAR MUCOSA

No significant change in ciliary activity was observed in the presence of solutions A through F or solutions J through O. However, ciliary activity was significantly reduced in the presence of solutions G through I, which contained 10^{-8} to 10^{-10} mol/L of PAF plus bronchoalveolar lavage fluid (BALF; Fig 2). This reduction in ciliary activity was observed within 5 minutes after the replacement, and further reduction was not found. The reduction was dependent on the concentration of PAF.

EFFECT OF INTRAVENOUS INJECTION

Otoscopic Examinations. No MEEs were detected in any animal of the saline group or the PAF group.

Ciliary Activity of Tubotympanum. At any time examined, ciliary activity in the 3 locations following intravenous injection of PAF was not significantly different from that following intravenous injection of saline solution (Fig 3).

Mucociliary Clearance of Tubotympanum. The MCTT of the PAF group at 10 minutes and 30 minutes was significantly longer than that of the saline group at the corresponding time. However, the MCTT of the PAF group 1 hour after intravenous injection was not different from that of the saline group at the corresponding time (Fig 4).

Morphology of Tubotympanum and Inner Ear. No pathologic findings were observed throughout the experimental period in the epithelium of the tubotympanum of the saline group or the PAF group. No inflammatory cell influx was observed in the tubotympanum of the PAF group (Fig 5). However, a partial loss of the inner periciliary fluid layer of the mucus

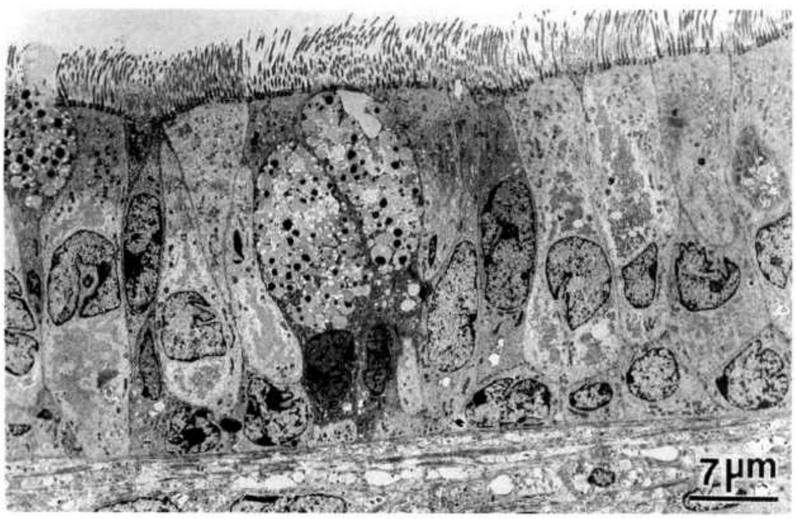

Fig 5. Transmission electron photomicrograph showing eustachian tube at 3 hours after injection of PAF. No pathologic findings are observed.

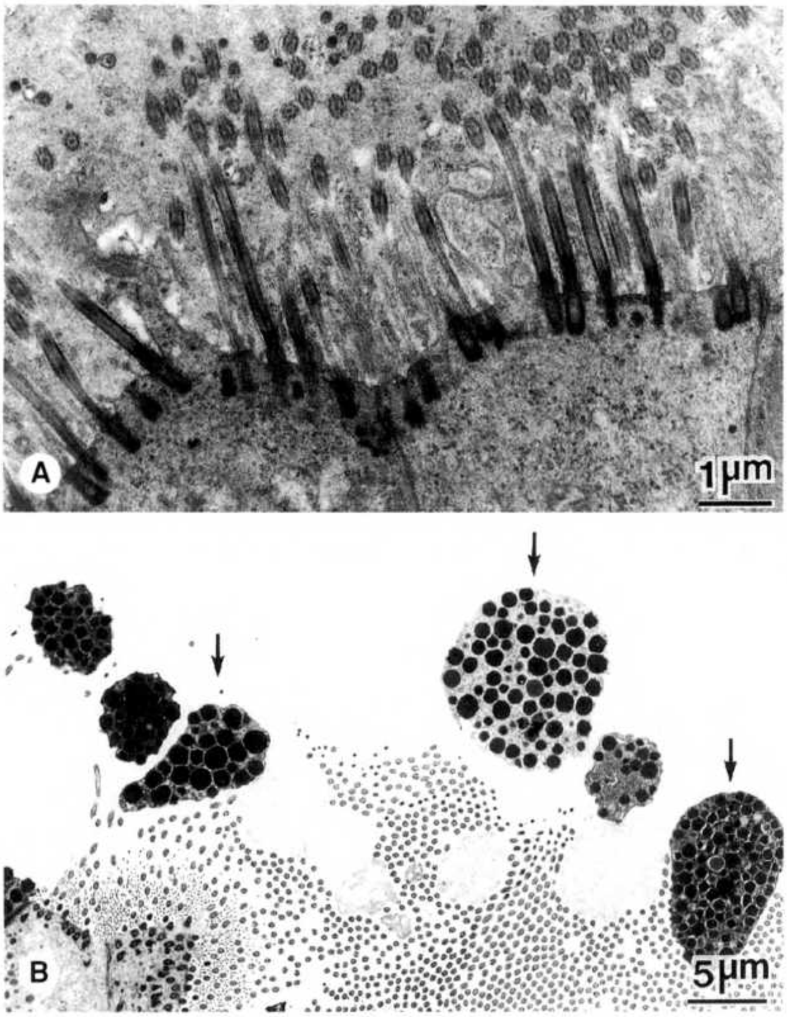

Fig 6. Transmission electron photomicrographs showing eustachian tube at A) 30 minutes and B) 10 minutes after injection of PAF. A) Partial loss of inner layer of mucus blanket is seen. B) Increased number of secretory granules (arrows) are discharged from goblet cells to lumen.

blanket (Fig 6A) and a number of secretory granules discharged from goblet cells (Fig 6B) were exclusively observed in the PAF group at 10 and 30 minutes after injection. On the other hand, some pathologic changes were sporadically observed at 10 and 30 minutes in the cochlea of the PAF group, which included cytoplasmic vacuolization of outer hair cells in the organ of Corti (Fig 7) and cytoplasmic ballooning (Fig 8A), as well as vacuolization (Fig 8B), of the stria vascularis. However, such morphologic changes were not found at 1 hour after intravenous injection of PAF.

EFFECT OF INTRATYMPANIC INJECTION

Otoscopic Examinations. No MEEs were detected in the saline group at any time examined. In the PAF group, however, the percentages of ears with MEEs were 40% and 20% at 3 days and 6 days, respectively. No animals of the PAF group demonstrated MEEs at 14 days.

Ciliary Activity of Tubotympanum. The ciliary activity in each location of the PAF group was significantly lower than that of the saline group at 3 days and 6 days postinoculation. However, the ciliary activity of every location recovered to the level of the saline group by 14 days (Fig 9).

Mucociliary Clearance of Tubotympanum. The MCTT of the PAF group was significantly longer than that of the saline group at 3 days and 6 days. However, the MCTT of the PAF group recovered to the level of the saline group by 14 days (Fig 10).

Morphology of Tubotympanum and Inner Ear. In the PAF group, accumulation of inflammatory cells, including eosinophils, was apparent in the middle ear mucosa as early as 3 days (Fig 11). Then, a significantly increased number of mast cells was observed in the middle ear mucosa. Degranulation of mast cells was found at 6 days. Epithelial cell injury including cytoplasmic vacuolization and sloughing of ciliated cells was sporadically observed in the tubotympanum at 6 days (Fig 12). However, few pathologic findings could be observed in the tubotympanum at 14 days postinoculation. On the other hand, no pathologic findings were observed in the inner ear, including the cochlea and the stria vascularis, of

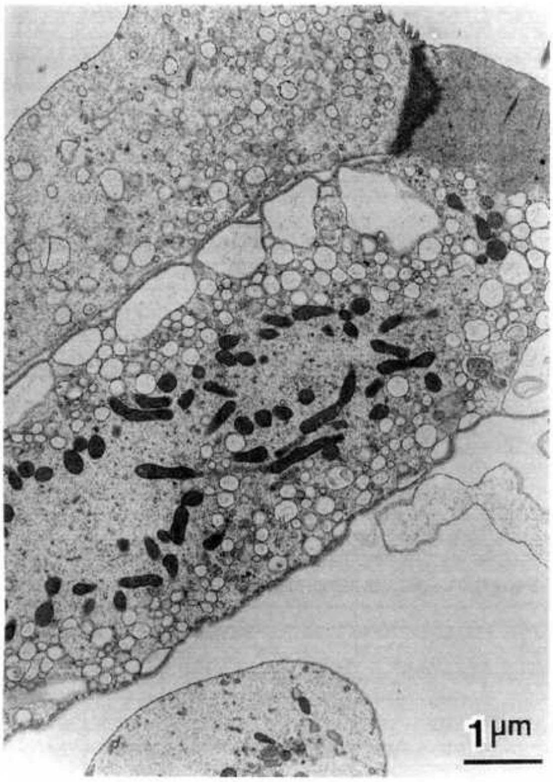

Fig 7. Transmission electron photomicrograph showing inner ear at 10 minutes after injection of PAF. Large number of small vacuoles are observed in cytoplasm of outer hair cells.

the PAF group (Fig 13).

DISCUSSION

The presence of PAF in MEEs of patients with OME was first examined by Van Cauwenberge and Bernstein[15] in 1987. Measurable levels of PAF were detected in 5 of 15 materials.[15] More recently, Furukawa et al[16] determined the concentration of PAF in MEEs obtained from 23 patients. The PAF was found mainly in mucoid MEEs, and the amount of PAF was higher in mucoid MEEs (3.55 ± 1.19 ng/mL) than in serous MEEs (0.44 ± 0.19 ng/mL). These reports suggest an important role of PAF in the pathogenesis of OME. In addition, Rhee et al[17] demonstrated that intratympanic injection of PAF caused persistent OME in chinchillas. However, the influence of PAF on the tubotympanum and the mechanisms by which PAF is involved in otitis media still remain to be conclusively elucidated.

The first aim of this study was to define the effect of PAF on the mucosa of the tubotympanum. The mucosa of the tubotympanum is equipped with a mucociliary system that consists of ciliary activity and a mucus blanket. Otitis media with effusion is accompanied by dysfunction of the mucociliary system.[8] Our first experiment focused on the effect of PAF on the in vitro middle ear ciliary activity. The effect of PAF on ciliary activity was previously studied in an in vitro experimental system by Ganbo and Hisamatsu.[18] They reported a ciliary inhibition by 10^{-6} to 10^{-10} mol/L of PAF in a dose-dependent as well as time-dependent fashion. However, a reduction in ciliary activity was not apparent within 1 hour after exposure to PAF. Platelet activating factor is quickly inactivated by acetylhydrolase in vivo.[19] Therefore, our examination was limited to 40 minutes after the contact with PAF. Our study has elucidated that PAF (10^{-8} to 10^{-10} mol/L) alone never reduced ciliary activity, but that the same concentration of PAF reduced ciliary activity in the presence of macrophages. Platelet activating factor is able to activate macrophages to produce superoxide.[20,21] Superoxide production by macrophages is therefore probably responsible for the ciliary depression observed in our experimental model.

Since macrophages are present in the middle ear mucosa, our in vitro study strongly argues in favor

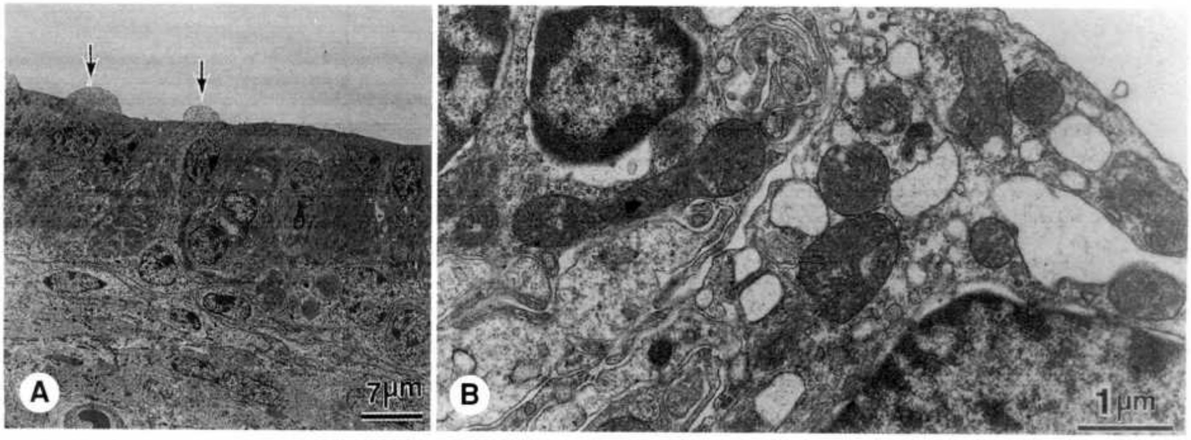

Fig 8. Transmission electron photomicrographs showing inner ear at A) 10 minutes and B) 30 minutes after injection of PAF. A) Cytoplasmic ballooning of marginal cells in stria vascularis (arrows) is sporadically observed. B) Cytoplasmic vacuolization of marginal cells in stria vascularis is sporadically observed.

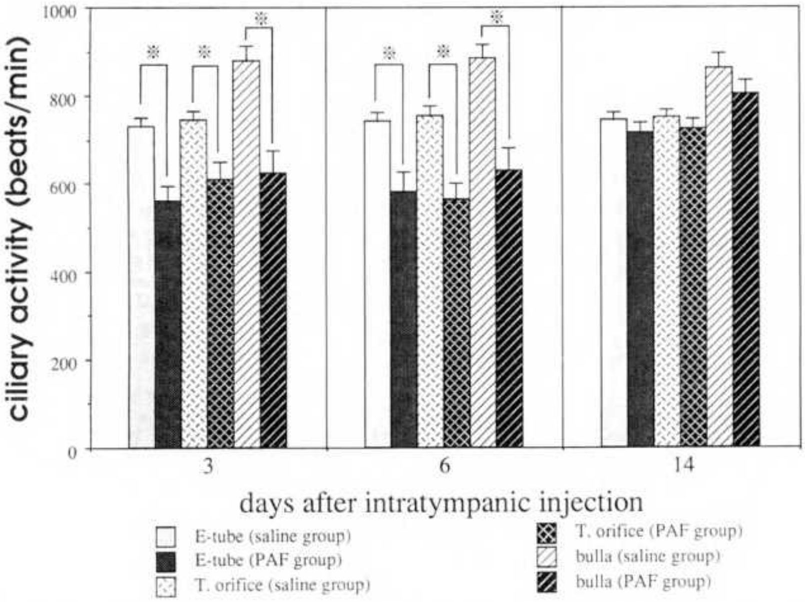

Fig 9. Ciliary activity following intratympanic inoculation of PAF. Ciliary activity of PAF group is significantly lower (※) at both 3 and 6 days after inoculation. However, ciliary activity recovers to level of saline group at 14 days.

of a possible role for PAF in the pathogenesis of mucociliary dysfunction in the middle ear mucosa. In vivo studies sometimes support hypotheses generated from in vitro systems, but sometimes, in vitro observations have little in vivo applicability. Hence, we decided to study the cilia response to PAF and the in vivo effect of PAF on the tubotympanum.

Intravenous injection of 10 ng of PAF has been shown to accumulate a significant number of eosinophils to the respiratory mucosa and induce epithelial cell injury in the respiratory mucosa.[22] In this study, however, the same dosage of PAF resulted in no eosinophil accumulation and no epithelial cell injury in the tubotympanum, whereas the magnitude and the mechanism of ciliary depression in the in vitro middle ear mucosa were quite similar to those established in the in vitro tracheal mucosa.[23] The most logical hypothesis to explain the discrepancy is that the target is not the tubotympanum, but the respiratory system, in the event of systemic PAF generation. In contrast, mucociliary dysfunction in the tubotympanum was apparent when PAF was directly introduced into the tympanic cavity. Such epithelial

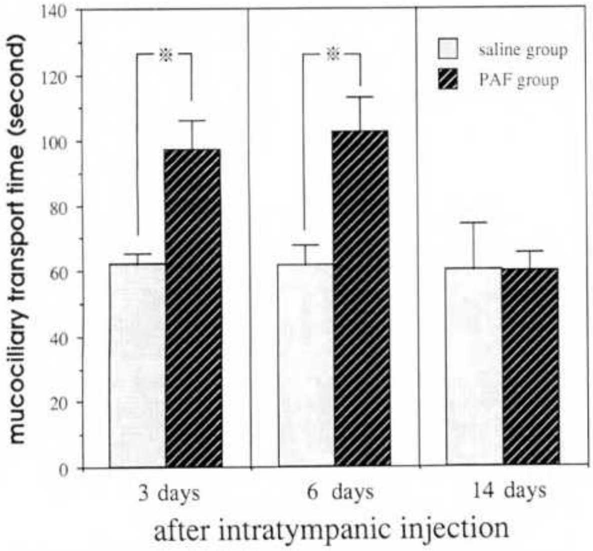

Fig 10. Mucociliary transport time (MCTT) following intratympanic inoculation of PAF. MCTT of PAF group is significantly longer (※) than that of saline group at 3 and 6 days. However, MCTT of PAF group recovers to level of saline group at 14 days.

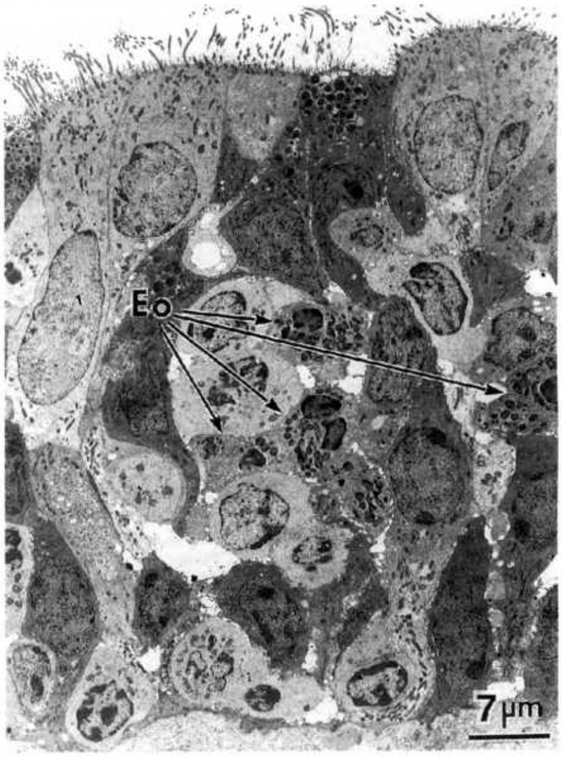

Fig 11. Transmission electron photomicrograph showing tympanic orifice at 3 days after inoculation of PAF. Number of eosinophils (Eo) have accumulated in epithelium.

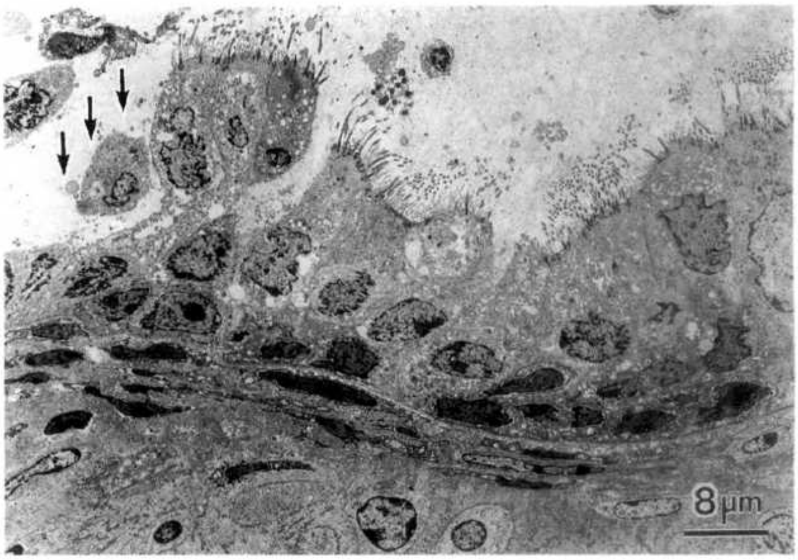

Fig 12. Transmission electron photomicrograph showing eustachian tube at 6 days after inoculation of PAF. Cytoplasmic vacuolization is observed in ciliated cells, and sloughing of ciliated cells (arrows) is sporadically observed.

cell injury was considered to be due to PAF-induced eosinophil influx and eventual eosinophil activation or inflammatory mediators and toxic proteins derived from degranulated mast cells and activated eosinophils. Therefore, our results suggest that the tubotympanum may not be a target organ, but that locally produced PAF can damage the mucosa of the tubotympanum, thereby leading to an inflammatory event and mucociliary dysfunction in the tubotympanum that could underlie middle ear diseases like OME.

Our present results demonstrated that under certain conditions, PAF could evoke inflammatory events in the middle ear. Inflammatory events and agents in the middle ear might spread to the inner ear through the round window membrane to develop an inner ear disorder. Indeed, secondary sensorineural hearing loss due to chronic suppurative otitis media has been documented in humans.[24,25] Additionally, sensorineural hearing loss can occur in experimental antigen-induced otitis media.[26] Taking these lines of evidence together, an increased level of PAF in the middle ear could theoretically induce an inner ear disorder, because PAF is a biologically potent mediator involved in allergy and inflammation. However, our study failed to reveal morphologic changes in the inner ear following the intratympanic injection of PAF. In contrast, intravenous injection of PAF resulted in apparent but reversible morphologic changes in the inner ear. Since intratympanic injection of PAF resulted in no middle ear disorders, the morphologic changes observed following intravenous injection of PAF are probably the result of anoxia produced by PAF-induced systemic hypotension.[27] This hypothesis is supported by the fact that after intravenous injection of PAF, the inner ear exhibited morphologic changes similar to those of systemic anaphylaxis or anoxia.[28,29] Platelet activating factor might damage the inner ear and thereby lead to some types of inner ear diseases that are not mainly due to the passage of PAF from the middle ear through the round window membrane, but the result of systemic anaphylaxis or anoxia.

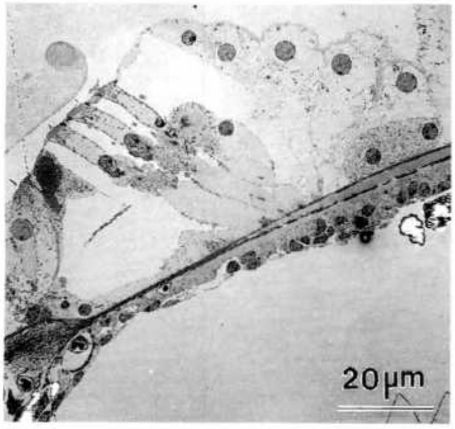

Fig 13. Transmission electron photomicrograph showing inner ear at 3 days after inoculation of PAF. No pathologic changes are observed.

In conclusion, PAF affects the mucociliary defense function of the tubotympanum and may be involved in the pathogenesis of some types of ear diseases, including OME and unexplained sensorineural hearing loss.

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KERATINOCYTE GROWTH FACTOR AND ITS RECEPTOR MESSENGER RNA EXPRESSION IN NASAL MUCOSA AND NASAL POLYPS

TOSHIO ISHIBASHI, MD
TADASHI TANAKA, MD
SHIN-ICHI ISHIMOTO, MD
KEN-ICHI NIBU, MD
KIMITAKA KAGA, MD
TOKYO, JAPAN

To examine the potential biologic role of fibroblast growth factors (FGFs) in nasal polyps and nasal mucosa during chronic inflammatory conditions, we investigated messenger RNA (mRNA) expression of three members of the FGF family — acidic FGF, basic FGF, and keratinocyte growth factor (KGF) — in nasal polyp tissues, as well as in hyperplastic nasal mucosa. Using the sensitive method reverse transcription–polymerase chain reaction (RT-PCR), we demonstrated that of the examined FGFs, KGF had the most abundant mRNA expression in nasal polyps and nasal mucosa. We also found that significantly higher levels of KGF mRNA were expressed in nasal polyps than in nasal mucosa, whereas mRNA expression of acidic FGF and basic FGF was relatively low in these tissues. In addition, we showed that KGF receptor mRNA was present in most of the nasal mucosa; however, none or little was expressed in nasal polyps. These results suggest that KGF might play an important role in nasal epithelial proliferation and that excessive synthesis of KGF in nasal polyp stroma may contribute to hypertrophy of the nasal mucosa in patients with chronic sinusitis associated with nasal polyposis.

KEY WORDS — fibroblast growth factor, keratinocyte growth factor, messenger RNA, nasal polyp, reverse transcription–polymerase chain reaction.

INTRODUCTION

Nasal polyps are rounded projections of edematous mucous membrane that arise from the ethmoid, sphenoid, maxillary, and frontal sinuses and eventually occlude the nostril completely. They may develop in association with chronic hypertrophic rhinitis, chronic sinusitis, or allergic rhinitis. Histologically, nasal polyps are characterized by proliferation and thickening of the mucosal membrane, with focal squamous metaplasia, hyperplasia of the mucous glands, and an edematous stroma with extensive neovascularization and infiltration by leukocytes, macrophages, and plasma cells.[1] Although the clinical and morphologic features of this disorder have been well characterized, the biologic roles of nasal polyps and their influence on nasal mucosa in chronic inflammatory conditions are poorly understood.

Fibroblast growth factors (FGFs) constitute a large family of structurally related multifunctional peptide mitogens of widespread tissue distribution that have mitogenic activity toward a variety of cells of mesenchymal, epithelial, and neuroectodermal origin.[2,3] They are involved in cell differentiation and proliferation, angiogenesis, embryogenesis, and tissue repair.[2,3] In view of the wide distribution of these factors, their biologic activities, and the histologic features of nasal polyps, there is considerable potential for their involvement in inflammatory and reactive changes of the nasal mucosa during chronic inflammatory conditions.

In this study, in order to clarify the role of FGFs in nasal mucosa, we investigated surgical specimens for messenger RNA (mRNA) expression of three members of the FGF family — acidic FGF, basic FGF, and keratinocyte growth factor (KGF) — in hyperplastic nasal mucosa and nasal polyps using the reverse transcription–polymerase chain reaction (RT-PCR). In an attempt to further characterize FGF-regulated functions in human nasal mucosa, we also evaluated mRNA expression of the receptors for these FGFs in these tissues. We demonstrated the expression of KGF and KGF receptor (KGFR) in nasal mucosa and increased expression of KGF in nasal polyps.

MATERIALS AND METHODS

Collection of Specimens and RNA Extraction. Eight nasal mucosa tissues from the inferior turbinate and 6 nasal polyp tissues were obtained from 14 adult patients with chronic sinusitis at the time of surgery for inferior turbinectomy or nasal polypectomy. All the specimens from the inferior turbinate were taken from clinically pathologic mucosa diagnosed as turbinate hypertrophy. No patient had under-

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From the Department of Otolaryngology, Tokyo University Branch Hospital (Ishibashi), and the Department of Otolaryngology, University of Tokyo (Tanaka, Nibu, Ishimoto, Kaga), Tokyo, Japan. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

CORRESPONDENCE — Toshio Ishibashi, MD, Dept of Otolaryngology, Tokyo University Branch Hospital, 3-28-6, Mejirodai, Bunkyo-ku, Tokyo 112, Japan.

PRIMERS AND PROBES USED FOR RT-PCR

mRNA	*Primer Pairs and Probes*		*Product Size (bp)*	*References*
KGF	5'	TCTGTCGAACACAGTGGTACCT	266	Finch et al[4]
	3'	GTGTGTCCATTTAGCTGATGCA		
	Probe	TCAGGACAGTGGCAGTTGGAATTG		
aFGF	5'	ATGGCTGAAGGGGAAATCACCAC	463 and 359	Zhao et al[5]
	3'	CAGAAGAGACTGGCAGGGGGAG		
	Probe	CCCAAACTCCTCTACTGTAGCAAC		
bFGF	5'	CTGTACTGCAAAAACGGG	349	Abraham et al[6]
	3'	AAAGTATAGCTTTCTGCC		
	Probe	CGGTCAAGGAAATACACCAGTTGGTA		
β-Actin	5'	CCCAGGCACCAGGGCGTGAT	263	Ponte et al[7]
	3'	TCAAACATGATCTGGGTCAT		
	Probe	CTGCTGACCGAGGCCCCCCTGACCCC		
KGFR	5'	TGCAGAAGTGCTGGCTCTGTTCAA	353	Miki et al[8]
	3'	GAGTTCATGGAGGAGCTGGACTCA		
	Probe	TGGCTGCTGAAGTCTGGCTTCTTG		
FGFR-2	5'	CTCTGCATGGTTGACAGTTCTGCC	255	Miki et al[8]
	3'	GAGTTCATGGAGGAGCTGGACTCA		
	Probe	TGGCTGCTGAAGTCTGGCTTCTTG		

5' — Forward primer; 3' — reverse primer. See text for abbreviations.

lying systemic disease such as allergic rhinitis, aspirin intolerance, mucoviscidosis, or Kartagener's syndrome. A section of tissue was dissected out, immediately frozen, and stored at –70°C for subsequent RNA isolation. The tissues were diced into slices on dry ice and then homogenized in Isogen (Nippongene, Toyama, Japan). Total RNA was precipitated with isopropanol and washed in 70% ethanol, then resuspended in TE buffer (10 mmol/L Tris, 1 mmol/L ethylenediaminetetraacetic acid, pH 7.6).

Reverse Transcription–Polymerase Chain Reaction. One microgram of total RNA from each sample was heated at 65°C for 5 minutes and cooled rapidly, then reverse-transcribed in a 10-μL volume containing 0.5 mmol/L dNTP (dATP, dCTP, dGTP, dTTP), 1 μg of random hexadenoxy-nucleotide primer, 40 U of RNase inhibitor (Boehringer Mannheim, Germany), 20 U of Moloney murine leukemia virus reverse transcriptase (Boehringer Mannheim), and reverse transcriptase buffer (supplied with enzyme) at 37°C for 60 minutes. The reaction was stopped by a 10-minute incubation at 80°C, and the samples were diluted with sterile water to a final volume of 50 μL. A 25-μL aliquot of PCR mixture contained 1.0 mmol/L of each primer set, 5 μL of complementary DNA (cDNA), 0.2 mmol/L dNTP, 10 mmol/L Tris–hydrochloric acid (pH 8.3), 1.5 mmol/L magnesium chloride, 0.01% gelatin, and 0.5 unit of Thermus Aquatics DNA polymerase (Taq polymerase; Boehringer Mannheim). The samples were overlaid with a drop of mineral oil to prevent condensation. Amplification was performed in a Minicycler (MJ Research, Watertown, Mass) using a "step-cycle" program set to denature at 92°C for 45 seconds, anneal at 60°C for 45 seconds, and extend at 72°C for 90 seconds. The cycle numbers were KGF, 30; acidic FGF, 45; basic FGF, 40; ß-actin, 30; KGFR, 35; and FGF receptor–2 (FGFR–2), 35. The RNAs from M426 human embryonic fibroblasts served as positive controls for acidic FGF and KGF, and RNA from B5 human mammary epithelial cells served as positive controls for KGFR. The RNAs from human placental tissues that had previously been shown to express basic FGF and FGFR-2 mRNA served as positive controls for basic FGF and FGFR-2. Identical reactions were used for negative controls, except that reverse transcriptase was omitted from the RT reaction (lane 16). Primer sequences were derived from separate exons of the gene sequence to ensure that amplification products of genomic DNA could be distinguished from products of reverse-transcribed mRNA. Nucleotide sequences for primer pairs and amplified fragments (base pair; bp) are shown in the Table.[4-8]

Southern Blot Hybridization. The PCR products were size-fractionated by electrophoresis through a 2.5% agarose gel, then visualized by ethidium bromide staining and transferred onto nylon membranes (Schleicher & Schuell, Keene, NH) according to the method of Southern.[9] The membranes were hybridized with nonradioactive oligo probes specific for a sequence internal to the primers used in the PCR amplification. Oligo probe sequences are shown in the Table. A digoxigenin (DIG) oligonucleotide 3'-end labeling kit (Boehringer Mannheim) was employed for nonradioactive hybridization of PCR

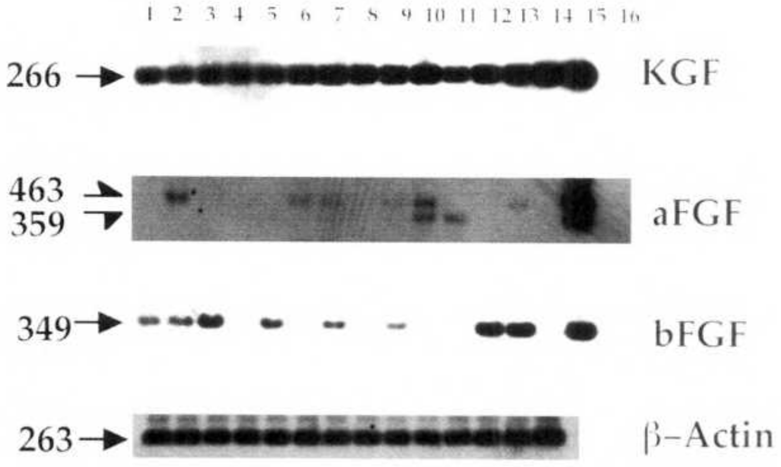

Fig 1. Expression of KGF, acidic FGF, and basic FGF mRNAs in hyperplastic nasal mucosa and nasal polyps as detected by Southern blot analysis of RT-PCR products. Total RNA isolated from each tissue was reverse-transcribed with random hexamers, and RT reaction was used in PCR to detect presence of indicated mRNA. Cycle numbers were KGF, 30; acidic FGF, 40; basic FGF, 40; and ß-actin, 30. Electrophoresed PCR products were blotted and hybridized with DIG-labeled oligonucleotides specific for sequence within primers. DIG-labeled probes were detected by enzyme-linked immunoassay using alkaline phosphatase–labeled anti-DIG antibody and chemiluminescence. RNAs from M426 human lung fibroblasts were used as positive controls for RT-PCR for acidic FGF and KGF (lane 15), and RNAs from human placental tissues that had previously been shown to express basic FGF served as positive controls for basic FGF (lane 15). Identical reactions were used for negative controls, with exception that reverse transcriptase was omitted from RT reaction (lane 16).

products according to the manufacturer's instructions. The DIG-labeled probes were detected by enzyme-linked immunoassay using an alkaline phosphatase–labeled anti-DIG antibody and chemiluminescence (Boehringer Mannheim). The filters were exposed to Kodak XAR films for 20 minutes to 2 hours at room temperature. Details of the hybridization and detection procedures are presented in the manufacturer's instructions for the DIG luminescent detection kit for nucleic acid.

Semiquantitative PCR for KGF and KGFR. Total RNA was isolated and reverse-transcribed as described above. Each cDNA sample was processed by PCR by the individual primer pair and sampled at 22, 27, 32, 37, and 42 cycles. Time kinetic studies were performed to evaluate the optimal number of amplification cycles. Based on this analysis, 27 amplification cycles for both KGF and β-actin and 32 amplification cycles for KGFR were found to be optimally within the logarithmic amplification range. Each sample was amplified in triplicate. The PCR amplification and Southern hybridization procedures are described above.

The exposed films were scanned into a Power Macintosh 8100/110 computer by an image scanner, and the intensity of the individual bands was quantified with NIH Image 1.57 software from the Division of Computer Research and Technology, National Institutes of Health, Bethesda, Md. Accordingly, the amount of hybridized probe was expected to be linearly related to the amount of PCR product. β-Actin was taken as an indicator of the actual amount of cDNA used in each PCR reaction. Therefore, the amount of target cDNA was quantified relative to the ß-actin level of each sample and was expressed in relative units of each gene. All data are expressed as mean ± standard error of the mean (SEM) from three independent experiments. Statistical significance was determined with the Wilcoxon-Mann-Whitney test. Differences between means were considered to be significant when p values were less than .05.

RESULTS

Messenger RNA Expression of FGFs in Nasal Polyps and Nasal Mucosa. To determine whether a distinct growth factor pattern occurs in nasal polyps and nasal mucosa, we investigated surgical specimens for mRNA expression of ß-actin and three members of the FGF family — acidic FGF, basic FGF, and KGF — in nasal polyps and nasal mucosa using RT-PCR followed by Southern blotting. As seen in Fig 1, the PCR products of KGF mRNA and ß-actin gave strong signals in every sample of nasal mucosa and nasal polyps after 30-cycle PCR amplification. Increased expression of KGF mRNA in nasal polyps was observed compared with that in nasal mucosa. Basic FGF transcript was undetectable after 30-cycle amplification, but was expressed in 6 of 8 nasal mucosa specimens and 3 of 6 nasal polyps after 40-cycle PCR amplification. One or two transcripts of acidic FGF were detected in 3 nasal mucosa specimens and 3 nasal polyps at very low levels after 45-cycle ampli-

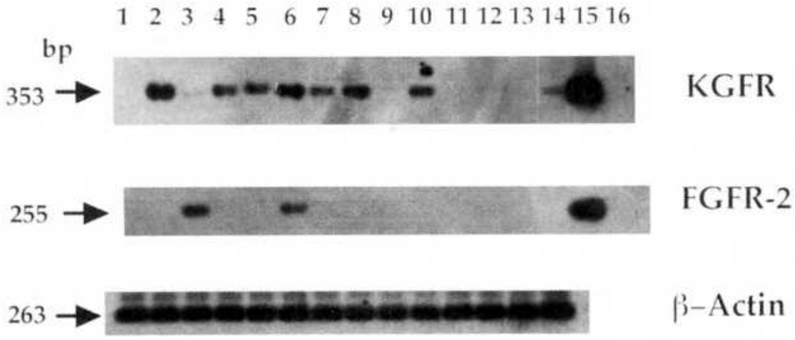

Fig 2. Expression of KGFR and FGFR-2 mRNAs in hyperplastic nasal mucosa and nasal polyps as detected by Southern blot analysis of RT-PCR products. PCR cycle numbers were ß-actin, 30; KGFR, 35; FGFR-2, 35; and ß-actin, 30. RNAs from B5 human mammary epithelial cells were used as positive controls for KGFR (lane 15). RNAs from human placental tissues that had previously been shown to express FGFR-2 mRNA served as positive controls for FGFR-2 (lane 15). Identical reactions were used for negative controls, with exception that reverse transcriptase was omitted from RT reaction (lane 16).

fication, although they had been undetectable after 35-cycle amplification. The RT-PCR of RNA from each positive control resulted in amplification of an appropriately sized fragment for each FGF in question (Fig 1, lane 15). No product was detected without reverse transcription (Fig 1, lane 16). These results demonstrated that the levels of KGF mRNA detected in nasal mucosa and nasal polyps were higher than those of acidic FGF or basic FGF.

Messenger RNA Expression of KGFR and FGFR-2 in Nasal Polyps and Nasal Mucosa. In an attempt to further characterize FGF-regulated functions in human nasal mucosa, we also evaluated mRNA expression of KGFR and FGFR-2 in these tissues. The KGFR, a spliced variant of FGFR-2/bek, was identical to FGFR-2 except for a strikingly divergent stretch of the second half of the third Ig-like domain. We designed upstream primers for the second half of the third Ig-like domain of KGFR and FGFR-2 and also designed a downstream primer in the juxtamembrane region, which had identical KGFR and FGFR-2 (see Table). Figure 1 demonstrates that KGFR mRNA was detected in 7 of 8 nasal mucosa specimens and 2 of 6 nasal polyps after 35-cycle PCR amplification. Decreased expression of KGFR mRNA in nasal polyps was observed compared with that in nasal mucosa. An FGFR-2 transcript was detectable in 2 of 8 nasal mucosa specimens, but was undetectable in 6 nasal polyps after 30-cycle amplification. The RT-PCR of RNA from each positive control resulted in amplification of an appropriately sized fragment for each FGF in question (Fig 2, lane 15). No product was detected without reverse transcription (Fig 2, lane 16).

Semiquantitative Assessment of KGF and KGFR in Nasal Polyps and Nasal Mucosa. Semiquantitative RT-PCR techniques were used to investigate KGF and KGFR mRNA levels from nasal polyps and nasal mucosa. Time kinetic studies were performed with the individual primer pair to evaluate the optimal number of amplification cycles. Based on this analysis, 27-cycle amplification for both KGF and ß-actin and 32-cycle amplification for KGFR were found to be optimally within the logarithmic amplification range. The PCR was performed under these reaction conditions, and the synthesized products were subjected to Southern blotting. Scanned intensities of the signals were integrated, normalized to ß-actin signals, and expressed in relative units (RU). This assay is sensitive and permits accurate compar-

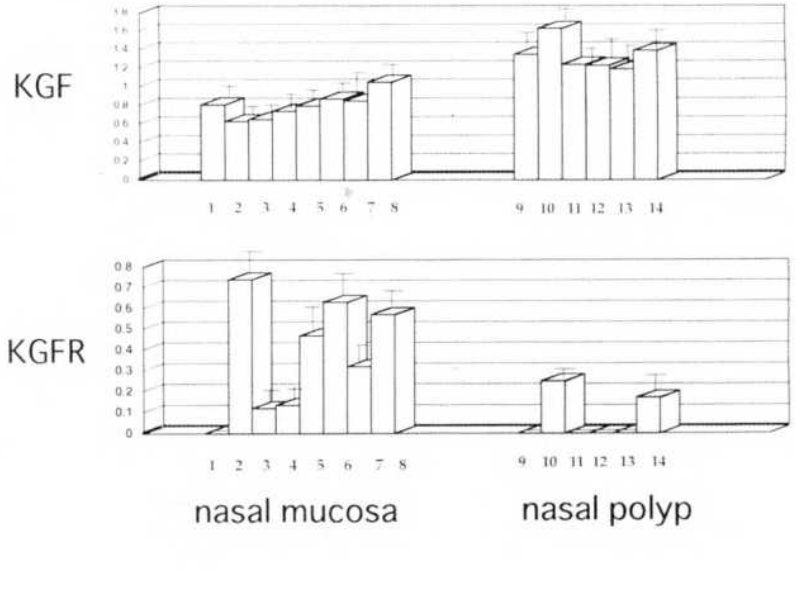

Fig 3. Semiquantitative analysis of KGF and KGFR mRNA in hyperplastic nasal mucosa and nasal polyps. Total RNA was isolated and subjected to RT-PCR followed by Southern blot analysis. PCR cycle numbers were KGF, 27; KGFR, 32; and ß-actin, 27 — which were found to be optimally within logarithmic amplification range by time kinetic studies. Exposed films were scanned into Power Macintosh 8100/110 computer by image scanner, and intensity of individual bands was quantified with NIH Image 1.57 software. Amount of target cDNA was quantitated relative to ß-actin level of each sample and expressed in relative units of each gene. All data are expressed as mean ± SEM from three independent experiments.

isons of mRNA expression in nasal polyps and nasal mucosa. Nasal mucosa demonstrated significantly higher levels ($p < .05$) of KGFR mRNA than did nasal polyps. In contrast, nasal mucosa demonstrated significantly lower levels ($p < .05$) of KGF mRNA than did nasal polyps (Wilcoxon-Mann-Whitney test; Fig 3).

DISCUSSION

Keratinocyte growth factor, also known as FGF-7, is a member of the FGF family.[4] It is synthesized and secreted exclusively by stromal cells in epithelial organs.[4] Unlike most other cytokines such as epidermal growth factor, transforming growth factor α, and other members of the FGF family that act on both epithelial and stromal cells, KGF specifically promotes proliferation in cells of epithelial origin as a paracrine effector.[10]

In this study, we used RT-PCR and Southern blot analysis to demonstrate that of the examined FGFs, KGF has the most abundant mRNA expression in nasal polyps and nasal mucosa. We also showed that KGFR mRNA is present in most nasal mucosa; however, none or little is expressed in nasal polyps. Keratinocyte growth factor has been identified as a paracrine mediator of proliferation in a variety of epithelial cells, including hepatocytes,[11] pancreatic epithelial cells,[12] mammary epithelial cells,[13] and skin keratinocytes.[14] However, expression of KGF and its receptor in nasal tissues has not, to our knowledge, been described previously. The results of this study suggest that KGF might be involved in the regulation of nasal epithelial cell growth.

The KGFR is closely related to the different forms of recently identified FGFRs.[15] Of these, FGFR-2, encoded by the bek gene, is most closely related to KGFR.[15] Both are derived from a common gene by mutually exclusive alternative mRNA splicing.[8] The difference is located in the C-terminal half of the third immunoglobulin-like domain.[8] These two receptor isoforms have strikingly different ligand-binding properties. The KGFR binds both KGF and acidic FGF with high affinity, but binds basic FGF with low affinity.[8] In contrast, FGFR-2/bek binds acidic and basic FGFs with high affinity, but not KGF.[8] We found that nasal mucosa and nasal polyps express very little or no acidic FGF; this finding suggests that acidic FGF is not so important in nasal mucosa. Although the majority of the nasal mucosa specimens from the inferior turbinate (6 of 8) and 3 of 6 nasal polyps accumulated basic FGF, only 2 inferior turbinate specimens and none of the nasal polyps expressed FGFR-2. These results raise the possibility that another receptor for basic FGF, such as FGFR-1, is operative in the nose.

It is interesting to note that significantly greater expression of KGF mRNA is present in nasal polyps than in nasal mucosa, whereas the expression of KGFR mRNA is decreased or absent in nasal polyps. These results suggest that excessive synthesis of KGF in nasal polyp stroma may contribute to hypertrophy of nasal mucosa in patients with chronic sinusitis with nasal polyposis. This might be a possible explanation for the fact that nasal polyps are often associated with chronic hypertrophic rhinitis.

Recent studies indicated that KGF mRNA expression is strongly induced in fibroblast cells by interleukin-1 (IL-1) stimulation.[16] Interleukin-1 plays an important role in a variety of biologic responses, including inflammation and wound healing.[17] In chronic sinusitis, nasal polyp stroma is infiltrated by a number of inflammatory cells, which would be one likely source of IL-1. The results of this study raise the possibility that IL-1 released by inflammatory cells could induce mRNA expression of KGF in nasal polyp stroma, which in turn might stimulate epithelial proliferation of nasal mucosa. Thus, KGF is likely to function as a paracrine mediator of mesenchymal-epithelial interactions in nasal epithelial cells during chronic inflammation.

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RHINOLOGY SYMPOSIA

The 18th International Symposium on Infection and Allergy of the Nose (ISIAN), the 4th Asian Research Symposium on Rhinology (ARSR), and the 3rd ASEAN Rhinology Group Congress (ARC) will be held in Manila, Philippines, February 14-18, 1999. For information, contact Gil M. Vicente, MD, Congress President, Dept of ORL-HNS, Jose Reyes Memorial Medical Center, Rizal Avenue Sta. Cruz, Manila, Philippines 1003; telephone (632) 743-6921; fax (632) 732-1077 or (632) 522-0946; e-mail isian@psohns.com; web site http://www.psohns.com/isian.

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EXPRESSION OF BASIC FIBROBLAST GROWTH FACTOR IN NASAL POLYPS

MICHAEL R. POWERS, MD
ZHENHONG QU, PHD
PHILIP C. LAGESSE, MD
JANICE M. LIEBLER, MD
MICHAEL A. WALL, MD
JAMES T. ROSENBAUM, MD
PORTLAND, OREGON

Basic fibroblast growth factor (bFGF) is a polypeptide that is mitogenic for a wide variety of cell types. We used Northern blot analysis and immunohistochemistry to determine if bFGF is expressed in the nasal polyp tissue; bFGF messenger RNA was detectable in the polyps examined by Northern blot analysis. Strong immunostaining for bFGF was found in blood vessels and along the basement membrane of the epithelial cell layers. Basal epithelial cells and some infiltrating mononuclear cells also stained for bFGF. Proliferating cell nuclear antigen colocalized with bFGF to basal epithelial cells, endothelial cells, and areas of focal epithelial metaplasia. The polyp tissue was double-labeled with a mouse monoclonal antitryptase, a specific mast cell marker, and anti-bFGF. A significant number (65% ± 19%) of the bFGF-positive mononuclear cells in the polyp tissues were positive for tryptase. These findings suggest that bFGF may contribute to the endothelial and epithelial proliferation in nasal polyp tissues and that mast cells are one source of this growth factor.

KEY WORDS — basic fibroblast growth factor, mast cell, nasal polyps, proliferating cell nuclear antigen, respiratory epithelium.

INTRODUCTION

Nasal polyps are inflamed and edematous tissue that arises from the nasal mucosa. In children, the occurrence of nasal polyps is often associated with cystic fibrosis (CF).[1] The histologic appearance of nasal polyps is characterized by the hyperplasia of mucosal epithelium and submucosal mucous glands with an underlying edematous stroma.[2] The polyps also have areas of proliferating blood vessels, focal fibrosis, and infiltrating inflammatory cells.[3] The cellular composition of the inflammatory cells in nasal polyps includes mast cells, macrophages, lymphocytes, and plasma cells.[4] Eosinophils are prominently found in non-CF nasal polyps.[4,5]

Basic fibroblast growth factor (bFGF) is a potent angiogenesis factor and is mitogenic for a wide range of cell types.[6] The extracellular matrix can act as a storage site for bFGF, associated with heparan sulfates in basement membranes.[7,8] Increasing evidence suggests that bFGF is involved in a variety of normal and abnormal biologic processes, including response to respiratory tissue injury.[9-11] Both bFGF and its receptor have been immunolocalized in the normal mucosal epithelium of human bronchial tissue.[12,13] In vivo, bFGF is mitogenic for respiratory epithelial cells, and the local administration of bFGF has also been reported to accelerate epithelial regeneration in a heterotopic rat tracheal isograft model.[14]

We have chosen to examine the expression of bFGF in nasal polyps to determine if this mitogenic growth factor may play a role in the growth of this inflamed upper airway tissue. Immunohistochemistry was used to evaluate the expression of bFGF and proliferating cell nuclear antigen (PCNA) in polyp tissue from CF and non-CF nonatopic patients. Mast cells were also evaluated as a potential source of bFGF in the polyp tissue. Polyp tissue was analyzed by Northern blot for the presence of bFGF messenger RNA (mRNA).

MATERIALS AND METHODS

Tissues. Nasal polyp tissues were obtained from patients who required removal of the polyps to relieve nasal obstruction. Polyp tissue was obtained from 10 patients with CF and 4 patients who were non-CF and nonatopic. For control tissue, nasal mucosa from the middle turbinate was obtained from 2 CF patients and 2 non-CF patients undergoing polypectomy. An additional control for upper respiratory tract tissue included archival normal bronchial tissue. Nasal polyps and mucosa were placed in normal saline on ice in the operating room and transported to the laboratory. The polyps and control nasal tissues were dissected and placed in 10% neutral

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From the Departments of Pediatrics (Powers, LaGesse, Wall), Ophthalmology (Powers, Rosenbaum), Cell and Developmental Biology (Qu, Rosenbaum), and Medicine (Liebler, Rosenbaum), Oregon Health Sciences University, Portland, Oregon. This work was supported by National Institutes of Health grant EY00318 and by a grant from the Gerlinger Foundation. Dr Powers was the recipient of a Physician Scientist Award from the National Eye Institute. Dr Rosenbaum was the recipient of a Senior Scholar Award from Research to Prevent Blindness.

CORRESPONDENCE — Michael R. Powers, MD, Dept of Pediatrics, Oregon Health Sciences University, 3181 SW Sam Jackson Park Rd, Portland, OR 97201.

buffered formalin overnight, then routinely processed and paraffin embedded. Polyp tissue was also snap frozen in liquid nitrogen for Northern blot analysis.

Antibodies. A mouse monoclonal anti-bFGF (148.6.1.1.1, gift of C. Hart, Zymogenetics, Seattle, Wash) was used in this study. Specificity of this antibody for bFGF has been previously characterized by immunohistochemistry and Western blot analysis.[15] A rabbit polyclonal anti-bFGF antibody was also used (BT-583, Biomedical Technologies Inc, Stoughton, Mass). According to the manufacturer, the antibody is specific for bFGF and does not cross-react with acidic fibroblast growth factor. A mouse monoclonal anti-PCNA was used as a marker of cellular proliferation (clone PC10, Signet, Dedham, Mass). A mouse monoclonal antitryptase was used as a specific mast cell marker (Dako Corporation, Carpinteria, Calif).[16] Specificity of staining was determined by using nonimmune immunoglobulins in place of the primary antibody. Control antibodies included nonimmune mouse IgG1 (MOPC21) for the monoclonal anti-bFGF and antitryptase (Sigma Chemical Co, St Louis, Mo). Nonimmune rabbit IgG was used as a control for the polyclonal anti-bFGF (Vector Laboratories, Burlingame, Calif). Nonimmune IgG2a (UPC10) was used as a control for the anti-PCNA (Sigma Chemical Co).

Immunohistochemical Staining for bFGF. Five-micron sections from the paraffin-embedded nasal polyps (N = 14) and control tissues were cut and mounted on poly-L-lysine–coated slides and stored at room temperature until stained. The sections were deparaffinized with xylene and hydrated in graded concentrations of ethanol and Tris-buffered saline (TBS; 50 mmol/L Tris, 0.15 mol/L sodium chloride, pH 7.5). Prior to incubation with anti-bFGF, sections were digested with 2 mg/mL hyaluronidase (Sigma Chemical Co) in 0.1 mol/L acetate buffer, pH 5.2, for 30 minutes at room temperature. The sections were rinsed with deionized water and then washed three times with TBS. Nonspecific binding sites were blocked with 2% normal horse or goat serum (Vector Laboratories), 0.1% bovine serum albumin, and 0.3% Triton X-100 in TBS for 30 minutes. The sections were incubated overnight at 4°C in a humidified chamber with the mouse monoclonal antibody at a 1:20,000 dilution (0.5 µg/mL) or the rabbit polyclonal anti-bFGF antibody at a dilution of 1:500 (2 µg/mL). Subsequent steps were carried out at room temperature. The tissue sections were washed three times in TBS and incubated with a biotinylated horse anti mouse IgG for the monoclonal antibody or a biotinylated goat anti rabbit IgG for the polyclonal antibody (1:200, Vector Laboratories). The sections were then washed three times in TBS, and incubated with an avidin-biotin–alkaline phosphatase complex for 1 hour (Vectastain ABC-AP Kit, Vector Laboratories). The sections were washed three times in TBS, and the antibody-antigen complexes were visualized with Vector Blue substrate (Vector Laboratories) or Fast Red substrate (BioGenex Laboratories, San Ramon, Calif) with 2 mmol/L levamisole (Vector Laboratories). The levamisole was included to block endogenous alkaline phosphatase. The tissue sections were counterstained with hematoxylin and covered with Crystal/Mount (Biomeda, Foster City, Calif).

Double Labeling of bFGF and PCNA. To determine the spatial correlation between bFGF and cell proliferation, polyp tissues were double-labeled for bFGF and PCNA. The polyp sections were initially labeled with the mouse anti-PCNA (PC10) at a dilution of 1:50. The PC10 clone was developed to identify proliferating cells in formalin-fixed, paraffin-embedded tissues.[17,18] The sections were deparaffinized and rehydrated as described in the previous section. Endogenous peroxide was quenched in hydrogen peroxide–methanol. To block nonspecific antibody binding, the sections were incubated with horse blocking solution as described above. The sections were incubated overnight with the anti-PCNA antibody at 4°C, washed with TBS, incubated with the biotinylated horse anti mouse antibody, washed with TBS, and then incubated with a horseradish peroxidase (HRP)–conjugated avidin biotin complex (Vector) for 40 minutes. The antibody-antigen complexes were visualized by incubation with an HRP substrate solution containing 0.5 mg/mL 3,3'-diaminobenzidine (DAB), 0.1% mol/L imidazole, and 0.03% hydrogen peroxide in PBS. The sections were then washed in TBS and stained with the mouse monoclonal anti-bFGF as described above, with Fast Red as the substrate. The tissue sections were then covered with Crystal/Mount (Biomeda) without counterstaining.

Double Labeling of bFGF and Tryptase. To determine if mast cells in the polyp tissues are a potential source of bFGF, the sections were double-labeled for bFGF and tryptase. The sections were first labeled for bFGF as described above, with the ABC-AP method with Vector Blue as a substrate. Potential nonspecific binding resulting from the first labeling was blocked with Avidin/Biotin Blocking Kit following the vendor's instruction (Vector). The sections were then incubated with antitryptase at a 1:200 dilution (0.5 µg/mL) overnight at 4°C and then incubated with biotinylated horse anti mouse and then avidin–fluorescein isothiocyanate at 1:100 for 1 hour at room temperature. Brief washes in TBS were per-

PATIENT DATA

Case	*Diagnosis*	*Sex*	*Age*	*No. of Polypectomies*	*Culture*
1	CF	F	10	1	PA, SA, H.flu
2	CF	F	31	1	PA
3	CF	F	15	2	PA
4	CF	M	14	3	SA, Strep
5	CF	F	13	1	PA
6	CF	F	12	4	PA, SA
7	CF	M	17	1	PA
8	CF	F	6	1	Strep
9	CF	F	20	1	PA
10	CF	M	24	1	SA
11	Non-CF	M	40	1	ND
12	Non-CF	F	26	1	ND
13	Non-CF	M	43	1	NG
14	Non-CF	F	13	1	ND

CF — cystic fibrosis, PA — *Pseudomonas aeruginosa*, SA — *Staphylococcus aureus*, H.flu — *Hemophilus influenzae*, Strep — *Streptococcus pneumoniae*, ND — not done, NG — no growth.

formed after each step. The double-labeled bFGF-tryptase sections were analyzed by light and immunofluorescent microscopy to determine the percentage of positive bFGF cells that coexpressed tryptase, as well as the percentage of tryptase cells that coexpressed bFGF. Sections were examined independently by two researchers (M.R.P., Z.Q.). Cells were counted at 400× magnification in four randomly selected areas on each slide (n = 8 cases) that included the epithelial mucosa and underlying stroma.

Northern Blot Analysis. Total RNA was isolated from frozen polyp tissue by the single-step guanidinium method.[19] The RNA was denatured and electrophoresed (20 µg per lane) through 1% agarose-formaldehyde denaturing gels with 0.25 µg/mL of ethidium bromide.[20] The RNA was transferred to a Nytran membrane (Schleicher & Schuell, Keene, NH) by capillary action in the presence of 10× SSC (1.5 mol/L sodium chloride, 0.15 mol/L sodium citrate). The Nytran blot was then cross-linked with ultraviolet light for 30 seconds. The blots were then prehybridized overnight in a buffer consisting of 50% formamide, 0.1% sodium dodecyl sulfate, 250 µg/mL heat-denatured herring sperm DNA (US Biochemical Corp, Cleveland, Ohio), 10× Denhardt's solution (0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin in diethyl pyrocarbonate–treated water), 50 µg/mL polyadenylic acid (Pharmacia, Piscataway, NJ), and 5× SSC at 60°C. The prehybridization buffers were then removed and replaced with fresh buffer supplemented with the ^{32}P-labeled bFGF complementary RNA (cRNA) probe. The human bFGF complementary DNA plasmid used to synthesize the cRNA probe was a generous gift of Dr Judith Abraham, California Biotechnology Inc, San Francisco, Calif. The blots were incubated for an additional 16 to 20 hours at 60°C. Blots were washed in 5× SSC (10 minutes, three times) at 62°C, in 0.1× SSC + 5 mmol/L ethylenediaminetetraacetic acid + 0.1% sodium dodecyl sulfate (120 minutes) at 62°C, and in 0.1× SSC (10 minutes) at 62°C. Blots were then autoradiographed at –70°C with Kodak XAR-5 film against an intensifying screen for 1 to 5 days. The size of RNA transcripts was determined according to the position relative to ribosomal RNA and RNA standards (Promega, Madison, Wis).

RESULTS

The Table shows the clinical characteristics of the 14 patients undergoing polypectomy for the treatment of nasal polyposis. Seven of the 10 CF patients were female, while in the non-CF group, 2 of 4 patients were female. The mean age of the CF patients was 16.2 years, while the mean age of the non-CF patients was 30.5 years.

Immunolocalization of bFGF. The nasal polyp tissues exhibited strong immunostaining for bFGF in the tunica media and tunica adventitia of blood vessels. The vessel staining was particularly intense along the submucosal region of the polyp (Fig 1A,B). Strong staining for bFGF was also localized along the basement membrane and basal cells in regions of normal mucosal epithelium in the polyps (Fig 1A,B). Intense immunostaining in a subset of mononuclear cells was apparent in the epithelial mucosa and polyp stroma (arrowheads). Both monoclonal and polyclonal bFGF antibodies gave an identical staining pattern, while nonimmune controls were negative (data not shown). Although the nasal polyps have variable histology with regard to degree of inflammatory cell infiltrate and submucosal edema, the strong immunoreactivity for bFGF in vessels, basal epithelial cells, basement membranes, and a subset of inflammatory cells was consistently found in both the CF and non-CF polyps. Figure 1C,D demonstrates the immunostaining of bFGF in control tissues. Nonpolyp nasal mucosa revealed light bFGF immunostaining along the epithelial basement membrane and basal layer of epithelial cells (Fig 1C). Strong immunostaining was seen in occasional mononuclear cells in the epithelial mucosa and submucosa. The normal bronchial tissues revealed moderate bFGF immunostaining along the epithelial basement membrane, while the basal layer of epithelial cells was lightly immunostained (Fig 1D). An occasional mononuclear cell and small vessel demonstrated a moderate amount of bFGF immunoreactivity in the submucosal region.

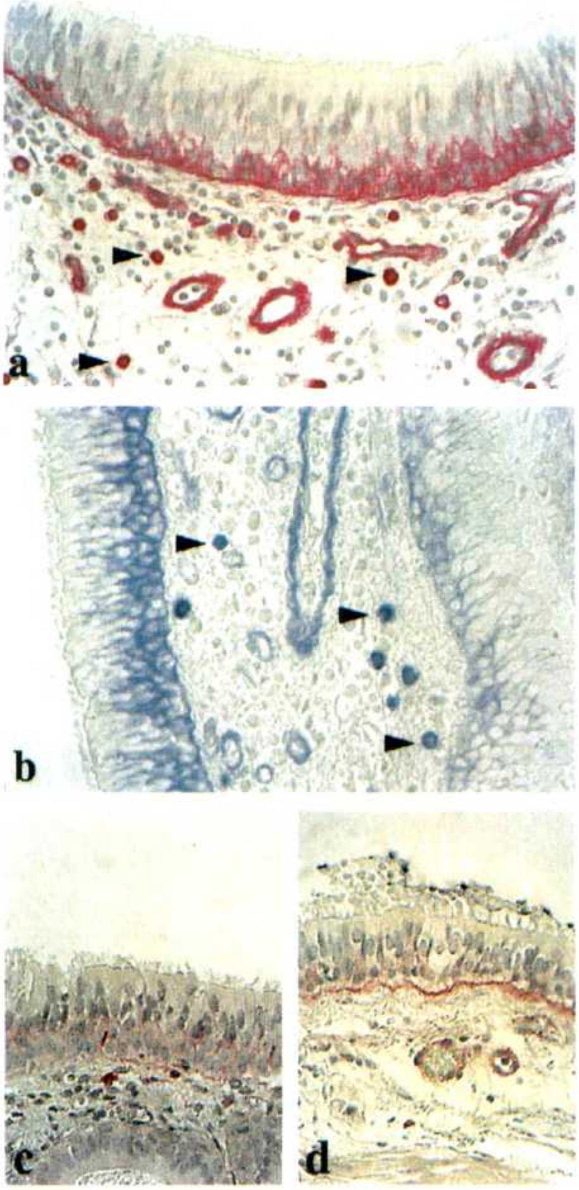

Fig 1. Immunostaining of a,b) nasal polyp and c,d) control tissues for basic fibroblast growth factor (bFGF; original ×400). a) (Case 1) With polyclonal anti-bFGF antibody, bFGF staining (red) is localized along basal epithelial cells and basement membrane of normal-appearing polyp mucosal epithelium (counterstained with hematoxylin). Blood vessels are intensely stained for bFGF in submucosal region. Subset of mononuclear cells in stroma and mucosa also exhibit intense immunostaining for bFGF (arrowheads). b) (Case 4) With monoclonal anti-bFGF antibody, bFGF staining (blue) is again localized along basal epithelial cells and basement membrane. Blood vessels and subset of mononuclear cells (arrowheads) also intensely stain for bFGF. c) Nonpolyp nasal mucosa immunostained with monoclonal anti-bFGF reveals light staining for bFGF (red) along basal epithelial cell layer and basement membrane, whereas occasional mononuclear cell is strongly stained (counterstained with hematoxylin). d) Normal bronchial tissue immunostained with monoclonal anti-bFGF (counterstained with hematoxylin). Light bFGF staining (red) is seen along basal epithelial cell layer and basement membrane. Vessels and occasional mononuclear cells exhibit moderate bFGF immunostaining.

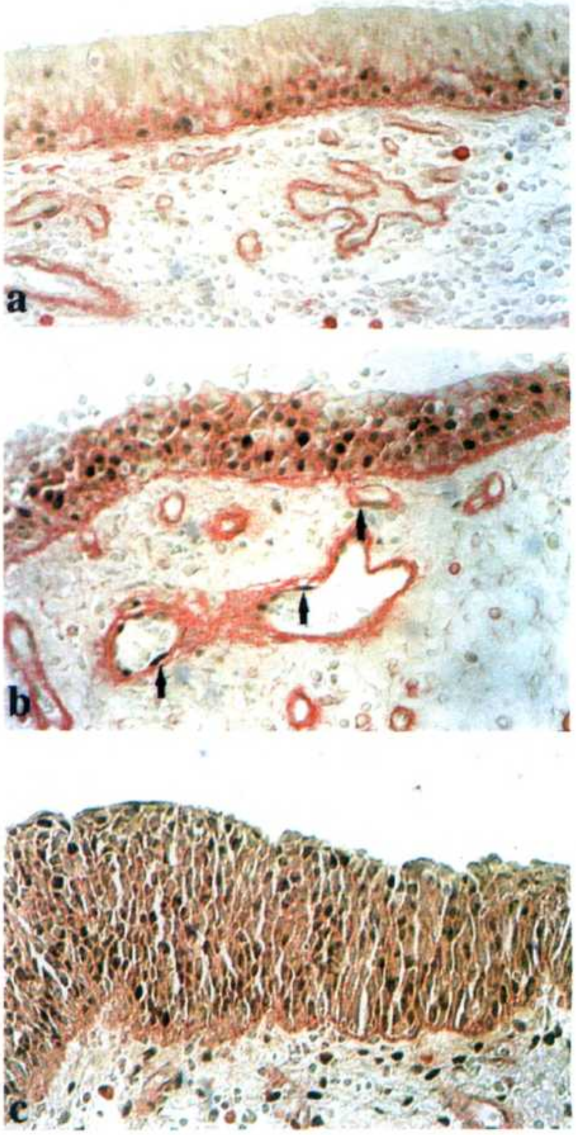

Fig 2. Double immunostaining of nasal polyp tissues for bFGF (red) and proliferating cell nuclear antigen (PCNA; dark brown; original ×400). a) (Case 1) Colocalization of bFGF and PCNA in basal and parabasal epithelial cells in normal-appearing mucosal epithelium. b) (Case 2) Extension of bFGF and PCNA staining from basal epithelial cells to include suprabasal cells in epithelial region of metaplasia. bFGF-positive blood vessels also demonstrate strong staining for PCNA (arrows) in endothelial nuclei. c) (Case 6) Epithelial region of extensive metaplasia again demonstrates colocalization of bFGF and PCNA in suprabasal cell layers. Note PCNA-positive fibroblastlike cells (brown) that are adjacent to several bFGF-positive mononuclear cells (red).

Double Labeling of bFGF and PCNA. The polyp tissues were subsequently double-labeled with anti-PCNA and anti-bFGF to determine if bFGF localized to areas of proliferating cells. The PCNA and bFGF were consistently colocalized in epithelial cells of the surface mucosa and glands. Figure 2A demonstrates bFGF-positive basal cells that are also la-

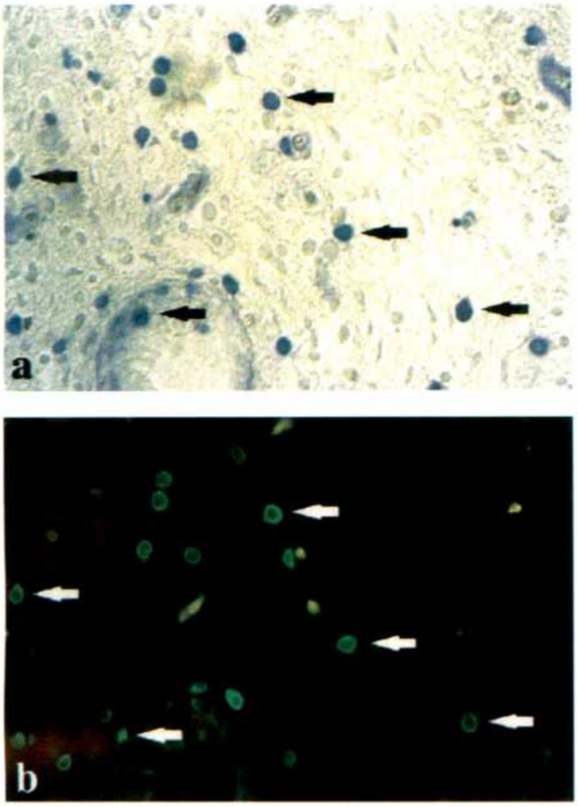

Fig 3. (Case 4) Double immunostaining of nasal polyp tissues for bFGF and tryptase (original ×400). Arrows indicate some of cells labeled by both anti-bFGF and antitryptase. a) There is intense immunostaining for bFGF (blue) in subset of mononuclear cells. b) Tryptase staining (green) is colocalized with majority of bFGF-positive cells in this section, as visualized by fluorescent microscopy.

beled for PCNA in normal-appearing mucosal epithelium.

Nasal polyps often exhibit areas of epithelial metaplasia. These regions of metaplasia are thought to be a response to local trauma and erosion. The bFGF immunoreactivity extended from the basal cells to the superficial cell layers in the areas of metaplasia. The regions of metaplasia also exhibited extensive labeling with PCNA beyond the basal cell layer to include several layers of suprabasal cells (Fig 2B,C). The bFGF-positive blood vessels also exhibited colocalization with PCNA (Fig 2B). Strong PCNA staining was found in fibroblastlike cells diffusely throughout the polyp stroma (Fig 2C).

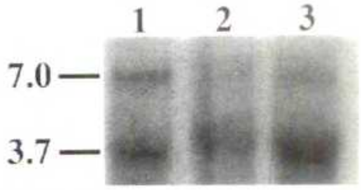

Fig 4. Autoradiograph of representative Northern blot hybridized with ^{32}P-labeled human complementary RNA for bFGF. Total RNA was isolated from polyp tissue, and 20 µg of RNA was run in each lane. RNA in lanes 1 through 3 was from case 2, case 5, and case 8, respectively. Major transcripts for bFGF messenger RNA were identified at 7.0 kb and 3.7 kb. Autoradiograph was exposed for 96 hours.

Double Labeling of bFGF and Tryptase. As described above, a subset of cells in the mucosa and stroma were intensely stained for bFGF. The morphologic appearance and tissue distribution suggested that some of these cells may be mast cells. Mast cells constitute approximately 10% to 15% of the inflammatory cells in nasal polyps. To investigate further the possible association of bFGF with mast cells, we double-labeled the polyp tissues with immunohistochemical and immunofluorescent staining. Polyp tissue immunostained with the monoclonal anti-bFGF revealed intense immunostaining (blue) in a subset of mononuclear cells (Fig 3A). A significant number of the bFGF-positive cells were also labeled with antitryptase (Fig 3B). Eight cases (6 cases CF, 2 cases non-CF) were double-labeled for bFGF and tryptase and examined as described in the Methods section. A significant number of bFGF-positive cells were also labeled for tryptase in all polyps examined (65.5% ± 18.7%). Of interest, the majority of tryptase-positive cells were double-labeled for bFGF (96.6% ± 3.3%).

Northern Blot. Northern blot analysis was performed to examine the nasal polyps for the presence of bFGF mRNA. Similar levels of bFGF mRNA expression were detectable in the nasal polyp tissues examined. The transcript sizes of 7.0 kb and 3.7 kb are similar to those reported for other tissues and cells such as synoviocytes (Fig 4).[21]

DISCUSSION

Nasal polyps have previously been used as an easily accessible tissue to examine cytokine expression in chronically inflamed upper airway tissues.[22,23] Our study was undertaken to examine if bFGF is present in nasal polyps, and therefore could potentially play a role in polyp tissue growth and subsequent nasal obstruction. Our results demonstrate the presence of both bFGF mRNA and immunoreactivity in nasal polyp tissues from CF and non-CF patients, suggesting that bFGF was expressed locally. That CF and non-CF polyps exhibited no apparent differences in mRNA expression or immunolocalization suggests that bFGF may be important in the pathogenesis of nasal polyposis regardless of the underlying cause. Widespread and strong immunostaining for bFGF was consistently present in the nasal polyp blood vessels; bFGF was also localized to blood vessels in control tissue, but the immunostaining was much lighter. The localization of bFGF in blood vessels in normal and diseased tissues has been previously described.[12,13,17] In normal vessels, the bFGF is most likely biologically unavailable, sequestered in the extracellular matrix.[24] However, under conditions of inflammation, such as that seen in the nasal polyp, the release of bFGF from extracellular stores may be enhanced as a result of the release of matrix-degrading enzymes from inflammatory cells.[24,25]

The localization of bFGF in the polyp and nasal mucosa is consistent with previous studies that have localized bFGF in a variety of epithelial tissues.[12,26] Strong bFGF immunostaining was exhibited in the basal and suprabasal layers of the polyp epithelium. In contrast, the normal control tissues only exhibited light bFGF immunostaining along the basal epithelium. A study by Coste et al[27] previously demonstrated that cell proliferation is increased in epithelium from nasal polyps as compared to nasal mucosa, particularly in the suprabasal area. Therefore, the intense coimmunolocalization of bFGF and PCNA along the basal and suprabasal epithelial cells suggests that bFGF could contribute to the proliferation of the epithelial cells and thus the growth of the nasal polyp mucosa.

A major finding in this study was the intense staining of bFGF in a subpopulation of infiltrating mononuclear cells. The vast majority of labeled mast cells (97%) were also labeled with bFGF in all polyps examined. The mast cells also were a large portion of the total number of bFGF-positive cells (65% ± 19%), though this finding varied somewhat from polyp to polyp. These results are consistent with previous work from our laboratory and others, in which mast cells were also demonstrated to be a source of bFGF in tissues with chronic inflammation and fibrosis.[28-31] Increased tissue mast cells have long been associated with disease processes that are characterized by chronic inflammation, neovascularization, and fibrosis.[32-34] That mast cells are significantly increased in nasal polyp tissues as compared to normal nasal mucosa suggests a key role for mast cells in the pathogenesis of nasal polyps.[35] The mast cells provide an additional source of bFGF that could contribute to the neovascularization, epithelial cell proliferation, and areas of focal fibrosis that are observed in nasal polyps. The tissue mast cells also contain heparin, which could increase the bioactivity of bFGF by protecting it from enzymatic degradation and acting as a cofactor for receptor binding.[36-38] Basic fibroblast growth factor lacks a conventional signal sequence, and the mast cell provides a potentially novel mechanism of bFGF secretion through degranulation.[30,39]

In summary, the present study demonstrates that bFGF mRNA and protein are expressed in nasal polyps. Because of the known properties of bFGF, this growth factor most likely plays a prominent role in the pathogenesis of nasal polyps, although this biologic effect is undoubtedly modulated by other growth factors and matrix proteins.[40,41] Additional studies will be required to further delineate the role of bFGF in nasal polyposis. Investigation into potential medical therapies of nasal polyposis might include specific inhibitors of bFGF.

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IMAGING CASE STUDY OF THE MONTH

VERTIGO PRODUCED BY PETROUS EXTENSION OF A RADIATION-INDUCED FIBROSARCOMA OF THE ANTERIOR SKULL BASE

NEIL BHATTACHARYYA, MD
HUGH D. CURTIN, MD
JOSEPH B. NADOL, JR, MD
WILLIAM W. MONTGOMERY, MD
BOSTON, MASSACHUSETTS

INTRODUCTION

Radiation-induced fibrosarcoma is a rare late complication of radiotherapy to the head and neck. We present a case of fibrosarcoma of the anterior skull base that occurred 17 years after radiotherapy for extensive inverting papilloma of the nasal cavity. Additional distinctive features of this case were vertigo and sensorineural hearing loss caused by lateral extension of the fibrosarcoma from the anterior skull base to the petrous bone and otic capsule, illustrating the potentially aggressive behavior of these lesions.

CASE REPORT

A 31-year-old man initially presented with complaints of bilateral nasal obstruction and recurrent frontal sinusitis. He was treated with nasal polypectomy and antibiotics, with no evidence of inverting papilloma in the pathologic specimen. He continued to have recurrent frontal sinusitis, and underwent successful osteoplastic frontal sinus obliteration. Two years later his symptoms returned, and he was found to have extensive polypoid disease of the right nasal cavity. A biopsy revealed inverting papilloma, leading to a right lateral rhinotomy and medial maxillectomy. Because of the extensive multifocal disease, residual papilloma was left at the anterior skull base. He was therefore referred for adjuvant radiotherapy to control the residual disease, and received a total of 6,000 cGy of external beam irradiation to the nasal cavity. Several follow-up examinations documented regression of the papillomata and remission of the frontal sinusitis.

The patient did well for 17 years, until he presented with recurrent right-sided epistaxis and a pulsatile sensation in the right side of the head. Physical examination along with magnetic resonance imaging (MRI) demonstrated a mass occupying the right nasal cavity and anterior skull base, as seen in Fig 1A. Enhancement of the lesion, as well as abnormal signal along the petrous ridge, is evident in Fig 1B. Computed tomography (CT) demonstrated the mass, as well as abnormal demineralization of the petrous apices bilaterally (Fig 2A). The patient then underwent a lateral rhinotomy and wide field resection of this mass, revealed by pathology to be a medium-grade fibrosarcoma; the surgical margins were negative.

Again, the patient did well, until he presented 1 month later with worsening right-sided pulsatile tinnitus, progressive sensorineural hearing loss, and severe vertigo provoked by coughing or Valsalva's maneuver. Repeat CT imaging demonstrated bilateral progression of the abnormal signal along the petrous apices posteriorly, as shown in Fig 2B. Erosion of the right otic capsule became evident, with destruction of the bone surrounding the cochlea. In consideration of the patient's diabetes and previous radiotherapy, the possible diagnoses included osteoradionecrosis, a low-grade osteomyelitis, and direct extension of the fibrosarcoma from the anterior skull base. The patient went to the operating room for a transmastoid biopsy of the right temporal bone, as well as a right labyrinthectomy because of his disabling vertigo, pulsatile tinnitus, and sensorineural hearing loss. Findings included tumor replacing the anterior external auditory canal, jugular foramen, posterior fossa plate, retrotympanic cells, promontory, and tegmen tympani. After the posterior aspect of the labyrinthine vestibule was surgically opened, palpation of the promontory revealed motion of perilymph, indicating diffuse involvement of the otic capsule by tumor. The patient tolerated the labyrinthectomy well, with resolution of his vertigo. Pathologic findings on the specimens from the otic capsule, anterior canal wall, and tegmen tympani were consis-

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From the Department of Otolaryngology–Head and Neck Surgery, Massachusetts Eye and Ear Infirmary and Harvard Medical School, Boston, Massachusetts.

CORRESPONDENCE — Neil Bhattacharyya, MD, Joint Center for Otolaryngology, 333 Longwood Ave (3rd Floor), Boston, MA 02115.

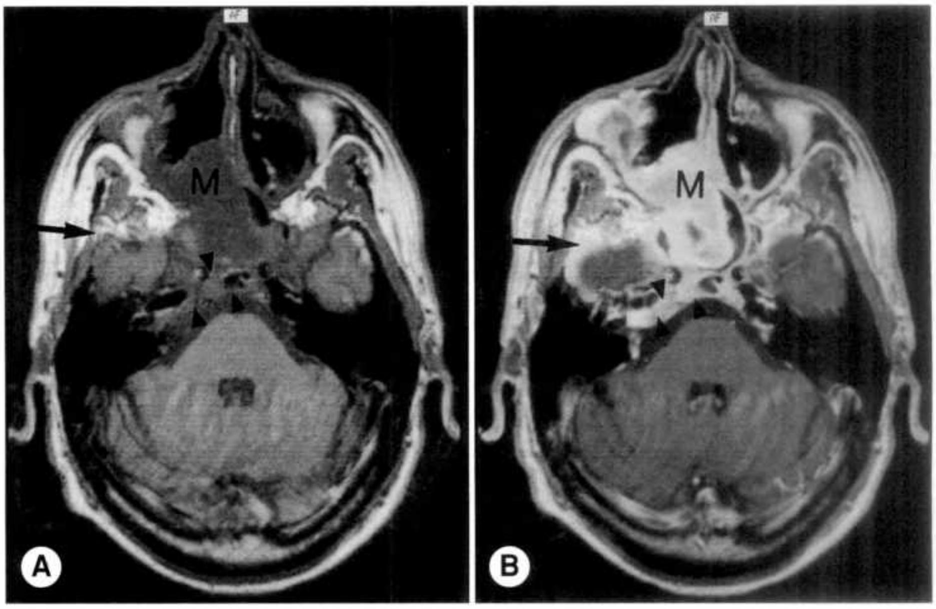

Fig 1. Axial Tl-weighted magnetic resonance images. A) Prior to contrast, showing large mass (M) in posterior nasal cavity and sphenoid sinus region. There is demineralization of clivus and petrous apex (arrowheads). Note abnormal signal of right greater wing of sphenoid (arrow). B) Postcontrast, demonstrating enhancement of nasal mass (M) and abnormal enhancement of clivus and petrous apex (arrowheads). Right greater wing of sphenoid also enhances abnormally (arrow).

tent with fibrosarcoma, identical to the previous sinonasal tumor histology. After six cycles of dacarbazine, doxorubicin hydrochloride, and ifosfamide followed by 4,500 cGy of radiotherapy, the patient's tumor has not increased in size on serial imaging studies, and he remains free of vertigo at 14 months' follow-up.

DISCUSSION

Previous studies have reported an incidence of 0.03% to 0.9% for radiation-induced sarcoma (RIS) in the head and neck, with a latency of onset of 10 to 30 years after treatment.[1,2] These sarcomas can be further subdivided histologically into osteosarcomas (50%), fibrosarcomas (25%), spindle cell sarcomas (8.5%), chondrosarcomas (7%), and other histologies (9.5%).[3] Criteria for the diagnosis of RIS have been proposed by Cahan et al[4] and have since been modified[1] to include 1) a sarcoma that develops in a previously irradiated field, 2) a latency period of

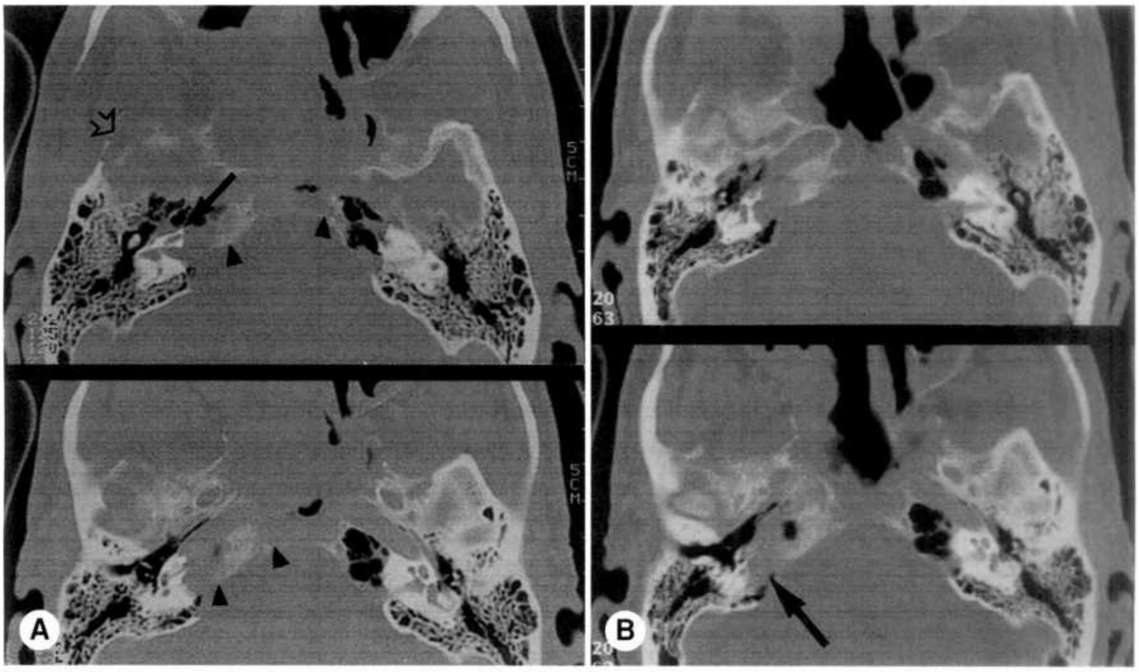

Fig 2. Axial computed tomography images. A) At level of right horizontal semicircular canal (top) and more inferiorly (bottom), demonstrating demineralization of petrous apex and clivus (arrowheads) by tumor. Note sharply demarcated demineralization of right otic capsule (black arrow), sparing cochlea. Compare to intact contralateral otic capsule. Open arrow designates demineralized greater sphenoid wing. B) At levels similar to A, 3 months later, demonstrating progressive invasion of right otic capsule (arrow) with complete demineralization of bone surrounding cochlea (compare to A). Left otic capsule remains preserved.

greater than 5 years from the radiation exposure to the occurrence of the sarcoma, and 3) a histopathology distinct from that of the original tumor. These three criteria were clearly fulfilled in this patient. Though the relationship between the dosimetry of the radiation and the induction of RIS is not completely clear, doses of less than 3,000 cGy are not likely to cause RIS. Notably, most cases of RIS occur after doses totaling approximately 5,500 cGy, which is comparable to most therapeutic doses for head and neck cancer.[1] While radiotherapy has been largely discontinued for benign conditions such as adenotonsillar hypertrophy and goiter, it remains an important modality in the treatment of carcinomas, sarcomas, and lymphomas of the head and neck. Although surgical excision remains the preferred form of therapy for sinonasal inverting papilloma, several studies have advocated radiotherapy for advanced, unresectable, and recurrent cases of sinonasal inverting papilloma.[5,6] As the follow-up in these studies is too short to assess the incidence of RIS after therapy, the relative risk of RIS should be considered when employing adjuvant radiotherapy for inverting papilloma.

The survival for radiation-induced fibrosarcoma of the head and neck, as extrapolated from data regarding RIS occurring in other sites, is generally poor, with survival rates ranging from 10% to 30% at 5 years.[7] This is significantly lower than the 66% 5-year survival that accompanies non–radiation-related sarcomas of the head and neck.[8] In cases of RIS that are resectable, surgical excision offers the best chance for cure, since chemotherapy results have been largely disappointing. Mark et al[9] elegantly compared the relative risks and complications of surgery, radiotherapy, and chemotherapy, and concluded that despite the risk of RIS, radiotherapy compares favorably with other treatment modalities.

Aside from the occurrence of an RIS after treatment for inverting papilloma, this case exhibits several other interesting clinical and radiographic features. One of these features is the sarcoma's pattern of extension to the middle cranial fossa and along the temporal bone, and the related manifestation of otologic symptoms. This tumor's infiltrative growth pattern along the superior surface of the temporal bone with destruction of the otic capsule is distinctly unusual, since the otic capsule of the temporal bone is generally resistant to penetration by tumor. Even in cases of primary sarcomas of the temporal bone, invasion of the otic capsule and presentation with sensorineural hearing loss and vertigo is rare.[10] In fact, the ability of the otic capsule to withstand invasion is radiographically used to distinguish tumors from fibrous dysplasia or Paget's disease within the temporal bone. Why this tumor extended laterally along the petrous apex without a significant mass effect is not clear; it may have been related to the blood supply remaining after previous radiotherapy to the anterior skull base. The radiographic pattern exhibited by the tumor's extension is also unusual: there was progressive demineralization of the petrous apex and otic capsule without an associated extraosseous component or change in the adjacent soft tissues. This radiographic pattern was felt to be more consistent with osteoradionecrosis of the temporal bone than with infiltration by tumor. The ongoing demineralization of the petrous bone by the tumor documented in Fig 2 may also have been related to the postirradiation characteristics of the skull base. Progressive tumor extension of this type — from the nasopharynx and anterior skull base to the otic capsule, resulting in otovestibular impairment — has not, to the best of our knowledge, been previously documented. Consideration should be given to the possibility of lateral extension of anterior skull base lesions to the petrous bone when symptoms of vertigo or hearing loss are encountered in patients with a history of therapy for such lesions.

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DELAYED FACIAL PALSY FOLLOWING UNEVENTFUL MIDDLE EAR SURGERY: A HERPES SIMPLEX VIRUS TYPE 1 REACTIVATION?

VIKTOR BONKOWSKY, MD
REGENSBURG, GERMANY

BERND KOCHANOWSKI, MD
REGENSBURG, GERMANY

JÜRGEN STRUTZ, MD
REGENSBURG, GERMANY

PAUL PERE, MD
MUNICH, GERMANY

WERNER HOSEMANN, MD
REGENSBURG, GERMANY

WOLFGANG ARNOLD, MD
MUNICH, GERMANY

In rare cases, a facial palsy appears a few days after uneventful middle ear surgery. The reason for this delayed palsy is unclear. One hypothesis is that it results from a reactivation of herpes simplex virus type 1 (HSV-1) in the geniculate ganglion of the facial nerve. From 1987 to 1996, in the course of over 1,800 middle ear operations, we observed 7 ipsilateral delayed facial palsies and investigated 5 of them using immunologic and virologic methods, including the polymerase chain reaction (PCR). We could detect HSV-1 genome with the nested primer PCR in the tongue swabs of 4 of the 5 examined patients with delayed facial palsy. The immunologic changes in these palsies are also compatible with a reactivation of HSV-1. We conclude that minimal stimulation of the facial nerve during middle ear surgery could result in a reactivation of HSV-1 in the geniculate ganglion, which may in turn lead to a facial palsy.

KEY WORDS — delayed facial palsy, facial nerve, geniculate ganglion, herpes simplex virus type 1 reactivation, mechanical stimulation, middle ear surgery, polymerase chain reaction.

INTRODUCTION

Facial palsy is a well-known but rare complication of middle ear surgery. In most such cases, the cause is traumatic and related to operative difficulties or anatomic anomalies. Such surgical trauma may result in immediate postoperative palsy. However, ipsilateral facial palsy may also occur several days after an otherwise successful and uneventful operation.[1] The reason for this delayed palsy is not clear.

Althaus and House[2] reported 5 cases of delayed post-stapedectomy facial paralysis that they considered to be related to edema of the nerve. Smith et al[3] mentioned the phenomenon of a Bell's-type facial nerve palsy occurring within a few days after an uneventful stapedectomy and speculated that it could be due to edema within the fallopian canal. Shea[4] also mentioned delayed facial palsy after stapes surgery and gave an incidence of about 1 in 1,000 cases. He speculated that the cause may be an immune response. Zohar and Laurian[5] also described a delayed facial palsy after uncomplicated stapes surgery. A diagnosis of coincidental Bell's palsy was made and the patient was treated with prednisone.

Adour et al[6] suggested that reactivation of herpes simplex type 1 virus (HSV-1) may play a major role in the pathogenesis of Bell's palsy, and more recent studies[7-9] strongly support this hypothesis. There is no doubt that HSV-1 is often present in a latent state in the human geniculate ganglion, and it is well known that surgical stress can reactivate herpes simplex virus in human ganglia; eg, a reactivation of HSV-1 is well documented after decompression of the trigeminal nerve root[10] or following section of the trigeminal sensory root.[11]

In the present work, we assumed that the hitherto unknown reason for delayed facial palsy occurring after uneventful middle ear surgery is HSV-1 reactivation in the geniculate ganglion of the facial nerve triggered by surgical stress, and we sought data that might support this hypothesis.

PATIENTS AND METHODS

From 1987 to 1996, in the course of over 1,800 middle ear operations, we observed 7 ipsilateral delayed facial palsies that fulfilled the following criteria: 1) uneventful middle ear surgery and 2) beginning of the palsy at least 48 hours after surgery.

Four of our patients were male and 3 female (Table 1), with ages ranging from 19 to 61 years (mean 40). In 2 cases a stapedotomy was performed, and in 5, a tympanoplasty. In all cases the operation was uncomplicated, with no special anatomic or surgical prob-

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From the Department of Otolaryngology, University Clinic Regensburg (Bonkowsky, Strutz, Hosemann), and the Institute of Medical Microbiology Regensburg (Kochanowski), Regensburg, and the Department of Otolaryngology, University Clinic Rechts der Isar, Munich (Pere, Arnold), Germany.

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CORRESPONDENCE — Viktor Bonkowsky, MD, ENT Department, University Clinic Regensburg, Josef Strauss Allee 11, 93042 Regensburg, Germany.

TABLE 1. PATIENTS WITH DELAYED FACIAL PALSY AFTER UNEVENTFUL MIDDLE EAR SURGERY

Patient	*Sex*	*Age (y)*	*Onset of Palsy (days postop)*	*Type of Operation*	*Anesthesia*
1	F	43	4	Tymp I	General
2	F	49	7	Stapedotomy	Local
3	M	19	2.5	Stapedotomy	General
4	M	61	6	Tymp III	Local
5	F	41	4	Tymp I	Local
6	M	35	5	Tymp I	General
7	M	32	5	Tymp III	General

Tymp — tympanoplasty (type I or III).

lems, except that the chorda tympani was cut in 2 cases. The onset of the facial palsy was between 2.5 and 7 days after surgery. Only 1 case of palsy (the first, in 1987) occurred after operation (tympanoplasty type 1) by an inexperienced ear surgeon. In the other cases, very experienced ear surgeons performed the operation. We investigated these palsies using immunologic and virologic methods.

Humoral Immune Response. Virus-specific IgG and IgM antibodies against HSV-1 were determined by enzyme-linked immunosorbent assay (ELISA) during the acute phase on days 1 to 3. The results were compared with a sex- and age-matched control group without a history of facial palsy or herpes labialis (n = 20), and antibody titers against HSV-1 were compared with those of patients with herpes labialis (n = 20). Herpes labialis is a well-known reactivation of HSV-1 with typical, easily diagnosed clinical symptoms. We therefore used this disease as a human model for definite reactivation of HSV-1.

T-cell Subpopulations. In each patient with delayed facial palsy, heparinized blood was taken in the acute phase on days 1 to 3, and T-cell subpopulations (CD3-, CD4-, and CD8-cells, activated CD8-cells, and natural killer [NK]–cells) were determined. The detection of T-cells has been described elsewhere.[12]

Polymerase Chain Reaction. In 5 of the 7 patients with delayed facial palsy (from 1991 to 1996) we took a swab from the tongue and searched for HSV-1 genome in the specimen using the polymerase chain reaction (PCR). Specimens from 10 healthy individuals without clinical or serologic signs of HSV-1 infection and from 10 post-tympanoplasty patients without delayed facial palsy were processed as a control.

Four PCR primers specific for the glycoprotein B of HSV-1 or HSV-2 were used. Primers 1 (5'-GTCAGAACTCGGAGG-3') and 2 (5'-CCATAAACTGGGAGTAGC-3') generated a 137 base pair (bp) product, and primers 3 (5'-GTGGTCTTCAAGGAGAAC-3') and 4 (5'-TGGCCGAACCACACCTGC-3') were used for nested PCR and resulted in an 89 bp product. Oligonucleotides (MWG Biotech, Ebersberg, Germany) were purified by high-performance liquid chromatography after synthesis. Thirty cycles in a Perkin Elmer 9600 thermal cycler (Perkin Elmer, Walterstadt, Germany) were performed as follows: denaturation at 94°C for 60 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 90 seconds. The final extension lasted for 7 minutes. The PCR mixture (50 µL) contained 200 µmol/L of each dNTP, 1.25 U Taq polymerase (Perkin Elmer), 0.1 µmol/L of each primer, and 5 µL of the DNA preparation in the PCR buffer of the GeneAmp kit (Perkin Elmer). In the nested PCR, 1 µL of the first-round PCR products was amplified under the conditions given above.

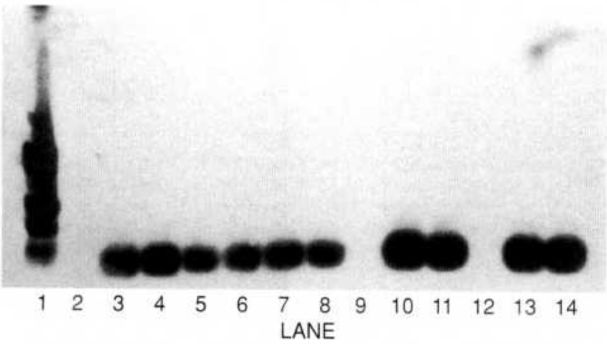
Herpes simplex types 1 and 2 were differentiated by treatment of the nested PCR product with the restriction enzyme Aat 2 (Boehringer Mannheim, Penzberg, Germany), which results in 2 fragments of HSV-1 of 62 and 27 bp length.

Material was washed from the swab tips with a small volume of 100 µL of 10 mmol/L Tris, 1 mmol/L ethylenediaminetetraacetic acid, pH 7.4 (triethanolamine buffer). DNA was isolated with the aid of silica gel membranes (Qiagen GmbH, Hilden, Germany) according to the instructions of the manufacturer. The DNA was eluted from the columns with triethanolamine buffer.

For DNA isolation, PCR, and product detection, the procedures of Kwok and Higushi[13] for preventing contamination were adhered to. Aerosol-resistant pipet tips were used (Biozym Diagnostik GmbH, Hess. Oldendorf, Germany), and all water was treated with diethyl pyrocarbonate (DEPC).

The herpes simplex virus–specific probe was generated by the amplification of HSV-1 and HSV-2 DNA containing plasmids with primers 3 and 4. Fifty micromoles digoxigenin-dUTP and 150 µmol dTTP instead of 200 µmol dTTP were used.[14] The resulting PCR product was separated on 1% agarose gel and isolated by the freeze-squeeze method.[15]

Agarose gel electrophoresis and Southern blot hybridization were performed as described previously.[14] In contrast to the published protocol, agarose gel electrophoresis of PCR products was performed with 4% agarose (NuSieve 3:1, Biozyme Diagnostik, Germany), and for Southern blot hybridization first-round PCR or nested PCR products were blotted onto the membrane for 1 hour with a vacuum blotter (Pharmacia, Freiburg, Germany). Detection was per-



Southern blot hybridization of second-round polymerase chain reaction products separated on agarose gel. Lanes 3 through 8 contain double values of 3 of positive patients, and lanes 10 and 11 and 13 and 14, of HSV-1– and HSV-2–positive controls, respectively. Lanes 2, 9, and 12 show negative serum samples as negative controls. DNA molecular weight marker (VIII, Boehringer Mannheim, Germany) in lane 1 indicates products of less than 147 base pairs.

formed with disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2'(5'-chloro)tricyclo[3.3.1.1]decan}-4-yl) phenyl phosphate (CSPD; Serva, Heidelberg, Germany) diluted 1:1,000 in 0.1 mol/L diethanolamine, 1 mmol/L magnesium chloride, pH 10.0 (assay buffer). The film was exposed for 2 hours or overnight.

RESULTS

Polymerase Chain Reaction. We could detect HSV-1 genome with the nested primer PCR in 4 of the 5 examined patients with delayed facial palsy (see Figure and Table 2). No HSV-1 genome was detected in the control specimens.

Humoral Immune Response. Immunoglobulin M antibodies against HSV-1 could be found in 1 of the 7 patients with delayed facial palsy. An increased IgG antibody titer in patients with delayed facial palsy after uneventful middle ear surgery was seen in 6 of the 7 patients, but this raised titer is not diagnostic of an acute infection or reactivation (Table 3).

A comparison of HSV-1 antibody titers between patients with delayed facial palsy after middle ear surgery and patients with herpes labialis demonstrated that the mean antibody titer (IgG) in delayed facial palsy after middle ear surgery was higher than in herpes labialis, a well-known and easily diagnosed reactivation of HSV-1 (Table 3).

TABLE 2. DETECTION OF HERPES SIMPLEX VIRUS TYPE 1 GENOME IN DELAYED FACIAL PALSY BY POLYMERASE CHAIN REACTION

Patient	*Type of Operation*	*Onset of Palsy (days postop)*	*PCR HSV-1 (Tongue)*
3	Stapedotomy	2.5	+
4	Tymp type III	6	–
5	Tymp type I	4	+
6	Tymp type I	5	+
7	Tymp type III	5	+

T-cell Subpopulation. In the acute phase, the proportion of T-cells was reduced, mainly due to a decrease in the CD4-cells leading to a CD4/CD8 ratio of 0.7 (normal 1.4 ± 0.3). The mean value of CD8-cells was in the normal range; however, the percentage of activated CD8-cells was 17.5% ± 5.2% and significantly greater than the percentage (<1%) found in normal persons. The percentage of NK-cells was also elevated in delayed facial palsy (26% ± 11%) versus normal persons (9% ± 4%). In order to better illustrate those findings, we report in detail 1 case of delayed facial palsy after uneventful middle ear surgery.

CASE REPORT

A tympanoplasty type I was performed on the left ear in a 41-year-old woman. Her past medical history was unremarkable, and routine laboratory blood findings were within normal limits. The surgical procedure consisted of endaural incision under local anesthesia, refreshing of perforation margins, elevation of the tympanomeatal flap, and anterior fascial underlay. The operation was uncomplicated, with no particular anatomic or surgical problems. On the fourth postoperative day, the patient complained of mild retroauricular pain, and on the next day a complete ipsilateral facial palsy was observed. A swab of the tongue was taken on the same day and HSV-1 genome could be detected with the PCR. The HSV-1 IgM titer was negative. The HSV-1 IgG titer was elevated (1:12,500), the CD4/CD8 ratio was reduced (1.1), and activated CD8-cells (44.6%) and NK-cells (25%) were increased.

On the same day that the facial palsy was diagnosed, we started antiviral therapy with intravenous acyclovir (20 mg/kg per day for 5 days) and steroid therapy with administration of prednisone 200 mg/d followed by tapering doses for 18 days. On the 11th day an improvement in the closing movements of the left eye could be observed. Good function of the

TABLE 3. COMPARISON OF HERPES SIMPLEX VIRUS TYPE 1 ANTIBODY TITERS IN PATIENTS WITH DELAYED FACIAL PALSY, PATIENTS WITH HERPES LABIALIS, AND NORMALS

	Delayed Facial Palsy (n = 7)	*Normals (n = 20)*	*Herpes Labialis (n = 20)*
IgM-positive	1 (15%)	0 (0%)	1 (5%)
IgG > 1:5,120	5 (71%)	1 (5%)	17 (85%)
IgG mean value	1:13,800	1:2,150	1:12,500

facial nerve (House II) could be documented on the 22nd day. Complete recovery of facial nerve function was achieved on the 34th day.

DISCUSSION

All the findings strongly support the hypothesis that a delayed facial palsy after uneventful middle ear surgery is due to mechanical reactivation (due to surgical stress) of HSV-1 virus. First, the detection of HSV-1 genome in the tongue swab of 4 of the 5 examined patients is proof that HSV-1 reactivation took place. Studies[7-9,16] have demonstrated that HSV-1 is in a latent state in the geniculate ganglion of the facial nerve, and it seems clear that mechanical disturbance of the chorda tympani and/or operating in close vicinity to the facial nerve could result in a reactivation of herpes virus in the geniculate ganglion. Passage of virus from the ganglion cells via intact sensory fibers (chorda tympani) to the periphery (tongue) occurs, and HSV-1 genome can then be detected in the tongue by PCR.

The mechanism of reactivation of HSV-1 due to middle ear surgery is very similar to reactivation of HSV-1 after decompression of the trigeminal nerve root.[10] During this operation, compressing blood vessels are microsurgically dissected from the trigeminal nerve root. No direct injury to the nerve occurs, but reactivation was detected in 28 (50%) of 56 patients.[10]

Both observations — reactivation of HSV-1 after uneventful middle ear surgery and reactivation of HSV-1 after decompression of the trigeminal nerve root — suggest that minimal stimulation or inapparent trauma to a nerve ganglion is sufficient to activate latent herpes simplex virus in humans.

These reactivated viruses lead to an immune reaction in the facial nerve and cause the delayed facial palsy.[17-19] The immunologic changes in this study are clearly compatible with this hypothesis. Changes in T-cell subpopulations in this study, including a low CD4/CD8 ratio and a higher percentage of activated CD8-cells and NK-cells, also support the hypothesis of a virus reactivation.[20]

In all cases of delayed facial palsy after uneventful middle ear surgery, we observed relatively high IgG antibody titers against HSV-1. These titers are not diagnostic of a reactivation and are not significant,[21] but a comparison of the titers between delayed facial palsy after middle ear surgery and herpes labialis — a sure reactivation of HSV-1 — shows nearly identical patterns.

Further, the IgG titers are about the same in delayed facial palsy after uneventful middle ear surgery and in Bell's palsy.[7,22]

According to Price,[23] 3 different trigger mechanisms for HSV-1 reactivation can be distinguished: 1) triggering in or near the ganglion, 2) skin stimuli (eg, ultraviolet light), and 3) systemic stimuli (eg, fever, stress, menstruation). In delayed facial nerve palsy, the trigger for reactivation is mechanical stimulation near the ganglion. In Bell's palsy, skin or systemic stimuli are probably the triggers for reactivation of HSV-1.[24]

The results of this study suggest that similiar to reactivation of herpes viruses after decompression of the trigeminal nerve root, minimal stimulation of the facial nerve during middle ear surgery could also result in a reactivation of herpes virus in the geniculate ganglion, thus leading to a facial palsy.

Fortunately, delayed facial palsy after uneventful middle ear surgery can be observed only in rare cases and not as frequently as HSV-1 reactivation after trigeminal nerve root decompression. There are several factors leading to the increased risk after trigeminal nerve root dissection, including the presence of many more cells, many more sensory nerve fibers, and a much larger skin innervation area in the trigeminal than in the geniculate ganglion. However, other unknown host or agent factors, such as differences in cell-mediated immunity, may play a decisive role.

These rare cases of delayed facial palsy following a therapeutically desired, well-tolerated stimulus provide unusual opportunities to study reactivation of latent virus in humans.

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MENIERE'S DISEASE

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CORRELATION OF ACOUSTIC THRESHOLD MEASURES AND SPIRAL GANGLION CELL SURVIVAL IN SEVERE TO PROFOUND SENSORINEURAL HEARING LOSS: IMPLICATIONS FOR COCHLEAR IMPLANTATION

ARMAGAN INCESULU, MD
ANKARA, TURKEY

JOSEPH B. NADOL, JR, MD
BOSTON, MASSACHUSETTS

In a temporal bone study of 26 ears from 13 patients who, in life, had severe sensorineural hearing loss, the segmental and total spiral ganglion cell (SGC) counts were correlated with hearing thresholds and with the difference between hearing thresholds in the two ears, the age at death, the duration of deafness, and the duration of hearing loss. A statistically significant correlation was found between the interaural differences in total SGC counts and the interaural difference in pure tone averages for 3, 4, and 5 frequencies. The total SGC count was higher in the ear with the better residual hearing in 11 of 12 cases. Approximately 41% of the variability in interaural difference in pure tone average was explained by the difference in SGC counts. The findings would suggest that in a given individual, selection of the ear with better residual hearing for cochlear implantation is likely to result in accessing a higher number of residual SGCs. This, in turn, may result in better speech recognition with the implant.

KEY WORDS — cochlear implant, human, patient selection, spiral ganglion.

INTRODUCTION

Handicapping hearing impairment affects 24 to 70 million people in the United States.[1] Although the exact number is not known, it has been estimated that of this group, as many as 1 to 2 million have profound hearing loss and obtain little or no benefit from conventional hearing aids.[2,3] Cochlear implantation can provide useful auditory cues in patients with severe to profound sensorineural hearing loss. In the past, candidacy for cochlear implantation was limited to patients with profound bilateral sensorineural hearing loss with a pure tone average (PTA) of 90 dB or greater and no open-set speech discrimination, and in general, the poorer-hearing ear was selected for implantation. However, with evidence that auditory performance among cochlear implant recipients may be better than that of hearing aid users in a comparable group of patients with severe to profound deafness with some residual hearing,[4-6] the auditory indications for implantation have been expanded. Thus, recent Food and Drug Administration guidelines have expanded the use of cochlear implants to include postlingually deafened adults with bilateral severe to profound sensorineural hearing loss with PTAs of 70 dB or higher and speech discrimination scores with sentence materials of 30% or lower in best aided conditions.[7,8]

In addition, some studies have demonstrated a modest but significant positive correlation between postoperative speech recognition scores in subjects using the implant and levels of preoperative residual hearing.[8-10] The reason for the correlation between residual hearing and auditory performance is unknown, but is assumed to be related to residual neural integrity.[10-12]

However, not all authors agree that residual hearing[13] or even residual spiral ganglion cell (SGC) count[14] is important for implant performance. In addition, there has not been conclusive evidence presented that better hearing thresholds are correlated with a higher SGC count in severe to profound deafness in humans.

The present study is an analysis of 26 ears of 13 patients who had residual hearing during life and would have been candidates for cochlear implantation under the newer and more liberalized criteria. In these patients, the segmental and total SGC counts from left and right ears were correlated with hearing thresholds and with the difference between hearing thresholds in the two ears. In addition, SGC counts were correlated with age at death, duration of severe to profound deafness, and duration of hearing loss.

MATERIALS AND METHODS

In the human temporal bone collection at the Mas-

From the Department of Otology and Laryngology, Harvard Medical School, and the Department of Otolaryngology, Massachusetts Eye and Ear Infirmary, Boston, Massachusetts. Dr Incesulu is currently at Portakal Cicegi Sokak, Ankara, Turkey. Supported by National Institutes of Health grant PO1 DC00361 and by a North Atlantic Treaty Organization Science Fellowship Grant to Dr Incesulu from The Scientific and Technical Research Council of Turkey (TUBITAK).

CORRESPONDENCE — Joseph B. Nadol, Jr, MD, Dept of Otolaryngology, Massachusetts Eye and Ear Infirmary, 243 Charles St, Boston, MA 02114-3096.

TABLE 1. CLINICAL DATA

Patient No.	*Otologic Diagnosis*	*Age at Death (y)*	*Duration of Hearing Loss*	*Duration of Severe to Profound Deafness*	*Interval Between Last Audiological Evaluation and Death*
1	Meniere's syndrome	65	34 y	24 y	24 y
2	Ototoxicity	69	1 y	1 y	1 mo
3	Meniere's syndrome	81	23 y	7 y	7 y
4	Otosclerosis	92	37 y	20 y	20 y
5	Hereditary syndrome*	65	? R 39 y L	7 y	6.5 y
6	Otosclerosis	88	25 y	4 y	2 y
7	Otosclerosis	71	53 y	21 y R 53 y L	24 y
8	Ototoxicity	19	2 mo	2 mo	2 mo
9	Congenital syphilis	91	>50 y	50 y	20 y
10	Labyrinthitis	70	60 y	28 y	1 mo
11	Mondini dysplasia	60	60 y	60 y	1.5 y
12	Usher's syndrome	65	65 y	65 y	26 mo
13	Alport's syndrome	14	2 y	2 mo	1 mo

*Autosomal dominant syndromal disorder with congenital cataracts and high-tone progressive sensorineural hearing loss.

sachusetts Eye and Ear Infirmary, there were 26 temporal bones from 13 patients who had residual hearing during life and would have been candidates for implantation under the liberalized criteria (PTA of 70 dB or higher and speech discrimination scores of 30% or lower). The otologic diagnosis, age at death, duration of hearing loss, and duration of severe to profound deafness are presented in Table 1.

All bones were fixed in Heidenhain-Susa solution, decalcified in trichloroacetic acid, and embedded in celloidin. The temporal bones were sectioned at a thickness of 20 μm in the horizontal plane. Every 10th section was stained with hematoxylin and eosin and mounted on a glass slide. The organ of Corti and spiral ganglion were graphically reconstructed by a method described by Schuknecht[15,16] and Otte et al.[17] All SGCs with visible nucleoli in every 10th section were counted with an ocular grid. Counts were multiplied by 10 to account for unmounted sections and also by a factor of 0.9 to account for doubly counted SGCs.[16] Spiral ganglion cells were counted for each of 4 segments of the spiral ganglion (segment 1, base of cochlea to 6 mm; segment 2, 7 to 15 mm; segment 3, 16 to 22 mm; and segment 4, 23 mm to apex). These counts were then added to obtain a total SGC count for each ear.

The bone conduction threshold — or the air conduction threshold, when the threshold exceeded the limits of bone conduction audiometry — was used to evaluate threshold at each frequency. There was no evidence of mixed loss, except in 3 cases (patients 6, 7, and 10).

Audiometric data gathered before 1964 were amended in accordance with the 1964 International Standards Organization standards. When the hearing threshold at a specific frequency exceeded the limits of audiometry, the threshold was estimated by adding 10 dB either to the stated audiometric limit at that frequency or to the measured audiometric threshold in the other ear.

Statistical analyses were performed with SAS software (SAS Institute, Cary, NC). Pearson correlation coefficients were used to examine the correlation between SGC counts in each of the 4 segments and the hearing results at each frequency (125 to 8,000 Hz), and between the total SGC counts and the PTA for 3 frequencies (0.5, 1, and 2 kHz), the PTA for 4 frequencies (0.5, 1, 2, and 4 kHz), and the PTA for 5 frequencies (0.25, 0.5, 1, 2, and 4 kHz). The correlation of SGC counts and the difference in PTA thresholds for 3, 4, and 5 frequencies between the right and left ears was evaluated by Pearson correlation coefficients and analysis of variance. The correlations between total SGC counts and age at death, duration of hearing loss (age at death minus age at onset of hearing loss), and duration of severe to profound deafness (age at death minus age at onset of severe to profound deafness) were also evaluated.

RESULTS

Pure tone audiometric data for the 13 subjects are presented in Table 2, and segmental and total SGC counts are presented in Table 3. There was no correlation between segmental SGC counts and hearing

TABLE 2. PURE TONE AUDIOMETRIC DATA

		Single Pure Tone Audiometric Results							*Pure Tone Averages*		
Patient No.	*Side*	*0.125 kHz*	*0.25 kHz*	*0.5 kHz*	*1 kHz*	*2 kHz*	*4 kHz*	*8 kHz*	*0.5, 1, 2 kHz*	*0.5, 1, 2, 4 kHz*	*0.25, 0.5, 1, 2, 4 kHz*
1	R	70	80	95	90	80	80	NT	88	86	85
	L	70	75	75	90	85	80	NT	83	82	80
2	R	NT	75	70	75	100	105	100*	82	88	85
	L	NT	60	65	65	105	100	100*	78	84	79
3	R	NT	75	80	80	75	75	85	78	78	77
	L	NT	80	85	90	85	100	100*	87	90	88
4	R	NT	105	105	110*	110*	105*	102*	108	108	107
	L	NT	100	105	110*	110*	105*	102*	108	108	106
5	R	NT	NT	110	115	NT	NT	NT	113	113	113
	L	NT	NT	120*	115	NT	NT	NT	118	118	118
6	R	NT	75	85	90	90	90	90	88	88.7	86
	L	NT	85	95	105	105	100	95	101	101	98
7	R	NT	70	75	80	80	85	80	78	80	78
	L	NT	75	80	80	80	90	85	80	82	81
8	R	NT	100*	100*	NT	NT	NT	NT	110	110	110
	L	NT	90	100	NT	NT	NT	NT	100	100	95
9	R	75	90	NT	NT	NT	NT	NT	90	90	90
	L	65	90	NT	NT	NT	NT	NT	90	90	90
10	R	NT	95	115	110	105	115*	100*	110	111	108
	L	NT	65	85	100	95	95	100*	93	94	88
11	R	100*	105	90	90	NT	NT	NT	90	90	95
	L	100*	115*	100*	100*	NT	NT	NT	100	100	105
12	R	NT	90	100	110	105	120	100*	105	109	105
	L	NT	100*	100	105	115	130*	100*	107	113	110
13	R	NT	100*	100*	100	105	105	110*	102	103	102
	L	NT	100*	100*	100	115*	115*	110*	105	108	106

Data are in decibels.
NT — not tested.
*Estimated.

thresholds at corresponding frequencies in the left and right ears (p > .1). Likewise, we did not find a significant correlation between total SGC counts and PTA thresholds for 3, 4, or 5 frequencies across ears. However, there was a statistically significant correlation between the interaural difference in total SGC counts and the interaural difference in PTAs for 3, 4, and 5 frequencies, particularly for 5 frequencies (p = .019; Table 4). That is, the total SGC count was higher in the ear with the better residual hearing in 11 of 12 cases. In 1 case (patient 9), there was no interaural difference in hearing. Approximately 41% of the variability in interaural difference in PTA was explained by the interaural difference in SGC count (see Figure). There was 1 case in which the correlation was reversed. However, this patient had a mixed conductive and sensorineural hearing loss due to otosclerosis, and hence, at frequencies with thresholds in the severe to profound range, the precise conductive component could not be determined. Thus, although the PTA was worse in the left ear, this loss included an unknown conductive component. In addition, the fact that the patient preferred the hearing aid performance in the left ear suggests that sensorineural hearing was better on the left. If this outlier was excluded, R^2 increased from .4070 to .5556 for the correlation between interaural difference in SGC count and interaural difference in PTA for 5 frequencies.

Pearson correlation coefficients were used to evaluate the correlation between total SGC counts and the age at death, the duration of hearing loss, and the duration of severe to profound deafness (Table 5). Negative correlations greater than .50 were found for all 3 variables, predicting that lower total SGC counts were found in older ears, in those with hearing loss of longer duration, and in those with severe to profound deafness of longer duration. The statistical significance of these correlations was strong (p < .03) for duration of deafness and age, and weaker for duration of hearing loss (p < .06).

DISCUSSION

Success in using a cochlear implant, as measured by speech recognition tests, may depend on many

TABLE 3. SEGMENTAL AND TOTAL SPIRAL GANGLION CELL COUNTS

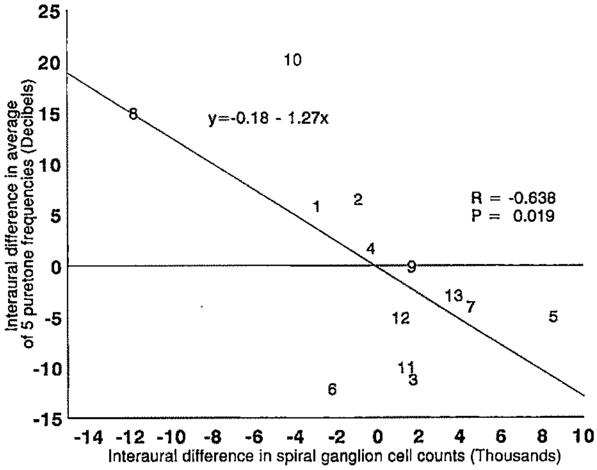
Patient No.	Side	*Spiral Ganglion Cell Counts* Segment 1	Segment 2	Segment 3	Segment 4	Total
1	R	1,143	3,231	2,637	3,150	10,161
	L	1,116	4,167	3,465	4,338	13,086
2	R	630	3,231	1,566	846	6,273
	L	612	3,564	2,322	648	7,146
3	R	1,863	6,156	4,680	4,275	16,974
	L	2,268	6,480	3,420	3,141	15,309
4	R	387	1,782	801	1,278	4,248
	L	603	2,160	1,080	846	4,689
5	R	2,205	7,128	4,239	3,852	17,424
	L	1,260	3,492	2,313	1,728	8,793
6	R	1,233	6,066	4,032	4,950	16,281
	L	1,215	5,913	4,599	6,705	18,432
7	R	738	3,735	2,232	2,466	9,171
	L	441	2,574	1,314	1,134	5,463
8	R	2,430	7,524	4,131	4,194	18,279
	L	5,103	11.061	6,345	7,488	29,997
9	R	1,260	2,790	1,683	2,061	7,794
	L	648	2,079	1,422	1,980	6,129
10	R	0	216	1,404	2,664	4,284
	L	693	2,349	2,259	2,979	8,280
11	R	1,611	5,463	2,988	1,746	11,808
	L	2,295	5,373	1,314	1,422	10,404
12	R	918	630	360	729	2,637
	L	0	450	513	481	1,444
13	R	4,743	13,329	8,073	5,139	31,284
	L	4,914	10,485	7,047	4,320	26,766

factors, including the integrity of the auditory nerve and central auditory pathways, age, cause of deafness, duration of deafness, hearing thresholds and dynamic ranges, design of the device, and capability of patients to function with degraded auditory cues.[10,18-20] Evidence suggests that some patients with cochlear implants demonstrate better speech recognition than patients using hearing aids in comparable groups with severe or profound sensorineural hearing loss but with residual hearing.[4-6] Further, residual hearing before implantation may predict modestly but significantly better speech recognition with a cochlear implant.[8-10] Improved auditory performance in individuals implanted with some residual hearing may be due to higher numbers of residual SGCs. Although this relationship has not been proven in humans, it is supported by implant performance data derived from animal studies. A number of animal studies have shown that psychophysical performance with a cochlear implant was related to the number of surviving auditory neurons.[11,21] Ears that had the lowest thresholds and greatest dynamic ranges with the implant also had the greatest number of surviving neural elements.

TABLE 4. CORRELATION BETWEEN INTERAURAL DIFFERENCES IN TOTAL SPIRAL GANGLION CELL COUNT AND INTERAURAL DIFFERENCES IN PTA

Variable	*Correlation*	*p*
Three-frequency PTA interaural difference	−.561	.045
Four-frequency PTA interaural difference	−.577	.038
Five-frequency PTA interaural difference	−.638	.019

PTA — pure tone average.



Approximately 41% of variability in interaural difference in pure tone average was explained by interaural difference in spiral ganglion cell count.

In this study we found a correlation between the interaural difference in SGC counts and the interaural difference in PTAs, suggesting that in a given individual the ear with the better residual hearing as estimated by PTA will have a higher residual SGC count. It is interesting that no significant correlation could be found between segmental SGC counts and residual hearing at corresponding frequencies, nor between total SGC count and PTAs across subjects. This is likely explained by the significant variation in SGC counts in subjects of different ages and different otopathologic disorders.[12,17]

These findings would suggest that in a given individual, selection of the ear with the better residual

TABLE 5. CORRELATION BETWEEN TOTAL SPIRAL GANGLION CELL COUNTS AND CLINICAL VARIABLES

	Right Ear		*Left Ear*	
Variable	*Correlation*	*p*	*Correlation*	*p*
Age at death	−.65	.016	−.72	.005
Duration of hearing loss	−.63	.028	−.70	.007
Duration of severe to profound deafness	−.53	.059	−.63	.020

hearing for implantation may result in better speech recognition. However, it is important to recognize that not all authors agree on the correlation between clinical performance with an implant and residual SGC counts.[13,22] The proof of this hypothesis must await direct comparison of postmortem SGC counts with premortem speech performance in large numbers of implant users.

This study also confirmed previous observations that the SGC counts were lowest in the temporal bones from individuals who had a long duration of hearing loss or a long duration of severe to profound deafness.[12,23] This finding is also consistent with the hypothesis that the number of residual SGCs is important for implant performance, since other studies have demonstrated a negative correlation between implant performance and duration of deafness.[10,11] However, several additional factors, such as dysfunction of remaining SGCs, long-term changes in the electrical properties of the cochlea, degeneration of higher auditory processing centers, or gradual loss of linguistic knowledge necessary for auditory processing, may also be important.[24]

Although cochlear implantation may result in loss of residual hearing, the selection of the better-hearing ear is likely to result in accessing more SGCs for electrical stimulation, which in turn may result in better auditory performance. Moreover, some studies have shown that electrical stimulation by a cochlear implant is not harmful to SGCs in humans[14,25] and animals,[26,27] and in fact, intermittent stimulation seems to have a protective effect resulting in a greater density of surviving SGCs in implanted and stimulated ears as compared to implanted and nonstimulated ears.[27,28] In contrast to the audiometric selection criteria used in the early years of cochlear implantation, it is now reasonable to consider implantation of the better-hearing ear, with appropriate consideration given to the clinical variables and personal priorities of each individual patient.

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CALCIUM PYROPHOSPHATE DEPOSITION DISEASE OF THE TEMPORAL BONE

JENNIFER A. JORDAN, MD
PETER ROLAND, MD
GUY LINDBERG, MD
DIANNE MENDELSOHN, MD

DALLAS, TEXAS

Massive calcium pyrophosphate crystal deposition is a rare disorder that may affect the temporomandibular joint and temporal bone. The diagnosis is difficult, and misdiagnosis as malignancy, particularly chondrosarcoma, is frequent. This can lead to unnecessarily aggressive management. The clinical, radiographic, and pathologic features of this entity are reviewed, and treatment options are discussed.

KEY WORDS — calcium pyrophosphate dihydrate deposition disease, pseudogout, temporal bone.

Calcium pyrophosphate deposition disease or CPDD was first described by Zitnan and Sitaj[1] in 1958, after they noted crystals of calcium pyrophosphate dihydrate in the synovial fluid of several patients. The term "pseudogout" was later ascribed to this condition by Kohn et al[2] as a result of the similarity of this entity to gout. Both gout and CPDD are characterized by crystal deposition and acute arthritis. In gout, the crystals are composed of uric acid and exhibit strong negative birefringence on examination with polarized light. In contrast, CPDD is characterized by calcium pyrophosphate crystals that appear weakly birefringent on examination with polarized light.[3] Calcium pyrophosphate dihydrate deposition disease has a distinct preference for fibrocartilage and only rarely involves hyaline cartilage. It most commonly manifests itself as an acute arthritis affecting the knee and wrist joints.[4] A much rarer form of CPDD, known as "tophaceous pseudogout," presents with massive crystal deposition, usually in a single joint, and most commonly affects the temporomandibular joint (TMJ).[5] Clinically, radiographically, and pathologically, this entity is frequently misdiagnosed because of its unusual presentation. We report a case of tophaceous pseudogout centered in the condylar fossa of the TMJ with extensive destruction of the temporal bone, which masqueraded as a chondrosarcoma of the temporal bone. All reported cases of CPDD involving the TMJ are also reviewed.

CASE REPORT

An 80-year-old, healthy, active man had a history of hypertension, hypercholesterolemia, and gout. He presented initially with an 11-month history of right-sided hearing loss that worsened suddenly following descent in an airplane flight. He denied any TMJ pain, trismus, jaw popping, or clicking. On physical examination, the only abnormality noted was a right-sided middle ear effusion. Audiologic testing at that time revealed only a mild right-sided conductive hearing loss and a type B tympanogram. The effusion was initially treated with a myringotomy, but rapidly reaccumulated.

Because his unilateral effusion reaccumulated rapidly, a computed tomography (CT) scan was obtained that revealed a mottled, expansile bony or cartilaginous proliferative mass involving the condylar fossa of the temporal bone extending to involve the floor of the right middle cranial fossa anteriorly to the greater wing of the sphenoid bone. The adjacent mandibular condyle cortex appeared uninvolved (Fig 1). These changes were felt to most likely represent Paget's disease or a tumor of cartilaginous origin such as chondroma or chondrosarcoma. Due to the patient's age, fibrous dysplasia was considered of low probability. A radioisotope bone scan was performed and this showed a single focus of abnormal uptake in the right temporal bone. Magnetic resonance imaging (MRI) was performed in the sagittal, axial, and coronal projections using standard spin echo as well as fat saturation techniques. This confirmed the presence of an approximately 4.5 × 4 × 3-cm, inhomogeneously enhancing mass centered at the right condylar fossa with a mass effect on the inferior temporal lobe. No abnormal marrow signal or enhancement was noted within the adjacent mandibular condyle (Fig 2). Fluid was seen to fill the right middle

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From the Departments of Otolaryngology (Jordan, Roland), Pathology (Lindberg), and Radiology (Mendelsohn), University of Texas Southwestern Medical Center, Dallas, Texas.

CORRESPONDENCE — Jennifer A. Jordan, MD, Dept of Otolaryngology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75235-9035.

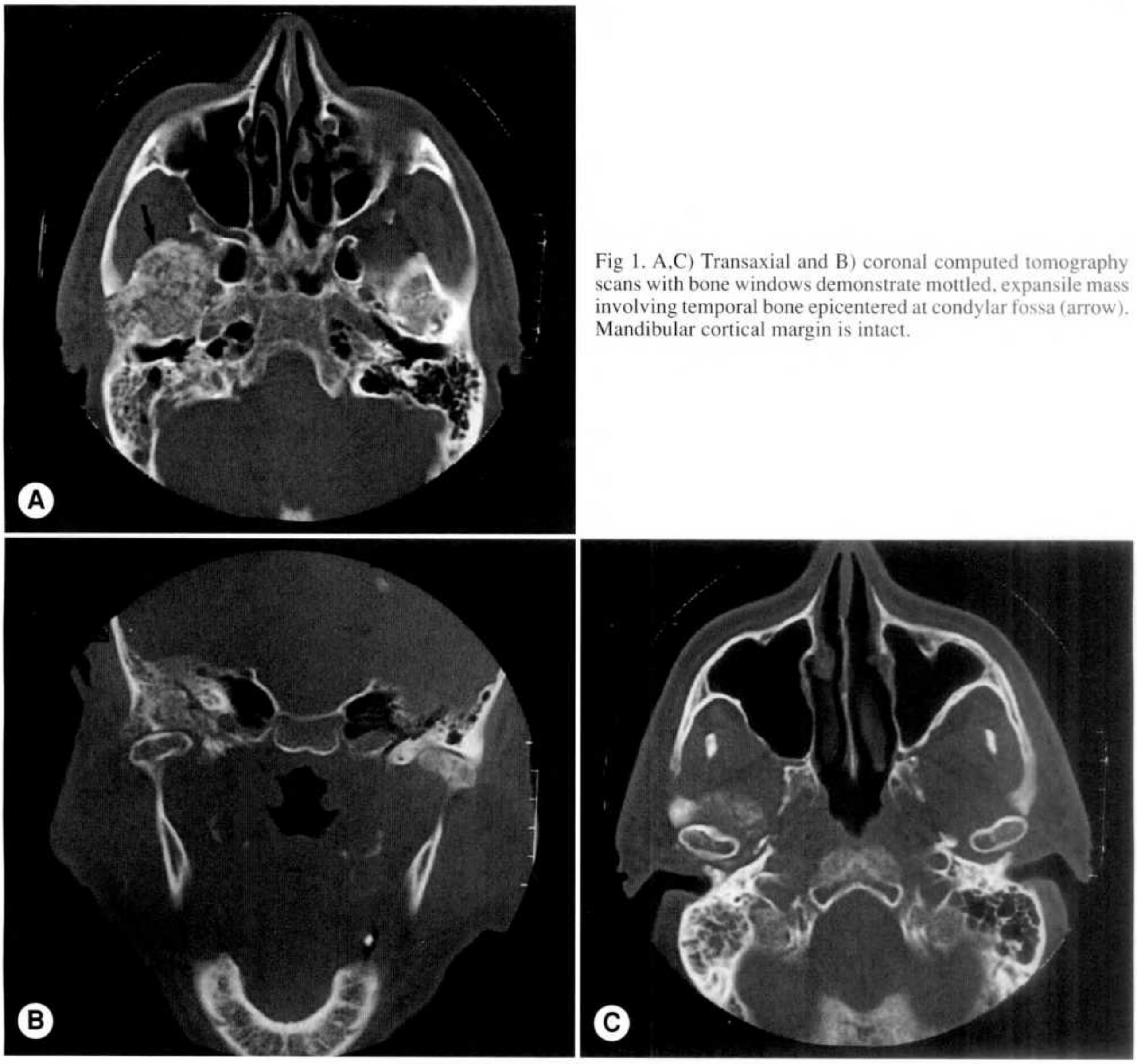

Fig 1. A,C) Transaxial and B) coronal computed tomography scans with bone windows demonstrate mottled, expansile mass involving temporal bone epicentered at condylar fossa (arrow). Mandibular cortical margin is intact.

ear and mastoid air cells due to resultant encroachment on the eustachian tube by the mass. A CT-guided biopsy of the lesion was subsequently obtained and was felt to represent a grade II or III chondrosarcoma.

Because of the patient's excellent health and level of activity, operative management was undertaken. "En bloc" resection of the mass was undertaken via an infratemporal postauricular approach to the middle cranial and infratemporal fossae with superficial parotidectomy and mobilization of the extratemporal portion of the facial nerve. Excision of the temporal squama and petrosa, floor of the middle cranial fossa, and middle ear space with resection of the mandibular condyle was required for complete removal of the mass. Immediate reconstruction of the defect was performed with a lateral thigh free flap.

Grossly, the lesion consisted of an 8-cm aggregate of friable tissue fragments that contained abundant punctate, white chalky material. The material extended onto the mandibular fossa of the temporal bone (Fig 3). Histologically, the tissue was dominated by large areas of deeply basophilic, calcified crystalline material. The crystals were needle- and rhomboid-shaped and with compensated polarized light microscopy exhibited a birefringence pattern characteristic of calcium pyrophosphate crystals. Focally, foreign body granulomatous inflammation was associated with the crystals. Surrounding the crystal deposits was cellular metaplastic chondroid tissue with moderately pleomorphic enlarged, hyperchromatic nuclei that simulated a chondrosarcoma (Fig 4).

Postoperatively, the patient was noted to have a House-Brackmann grade IV facial nerve paresis that

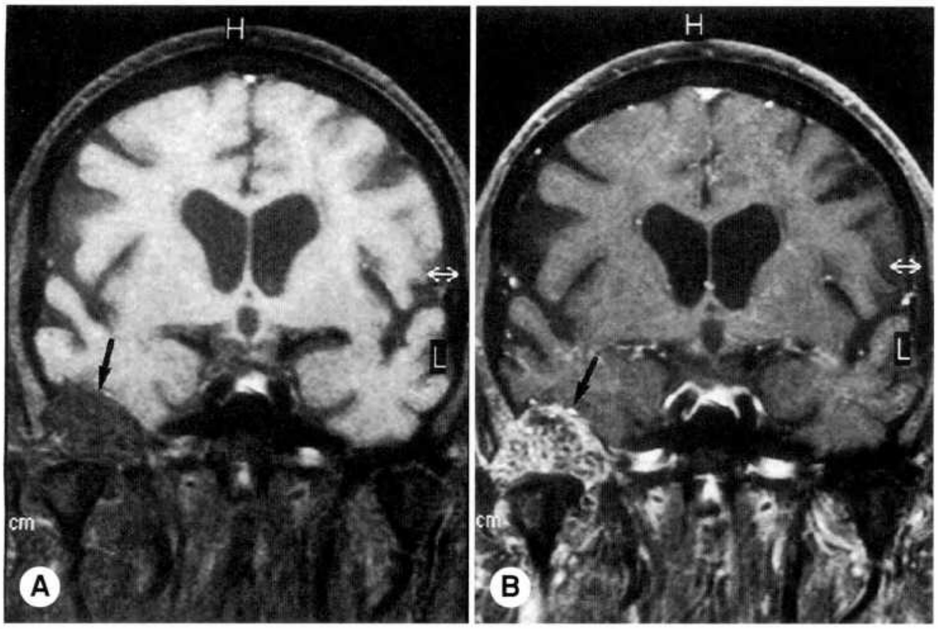

Fig 2. T1-weighted images A) before and B) after administration of intravenous gadolinium–diethylenetriamine pentaacetic acid demonstrate inhomogeneously enhancing mass involving skull base (arrows). Mass indents undersurface of adjacent right temporal lobe, with no involvement of adjacent mandibular condyle.

progressed to complete paralysis by postoperative day 2. His hospital course was uncomplicated and he was discharged to home on postoperative day 8. Postoperatively, the patient has done well and has no evidence of recurrent disease.

DISCUSSION

Calcium pyrophosphate dihydrate deposition disease has many different forms that contribute to the difficulty in making a diagnosis. Several classification schemes have been postulated based on the clinical pattern of the arthritis.[6,7] In general, there are acute and chronic forms of the disease. The acute arthritic form, which most commonly affects the knee, is characterized by an acute joint effusion with calcium phosphate crystals that lasts approximately 10 days and is often precipitated by stress. The acute processes infrequently involve the TMJ. The chronic forms are less common and often indistinguishable from osteoarthritis. Tophaceous pseudogout is one of the least common types of chronic disease, with only approximately 35 reported cases, 19 of which involved the TMJ (Table 1[5,8-20]).

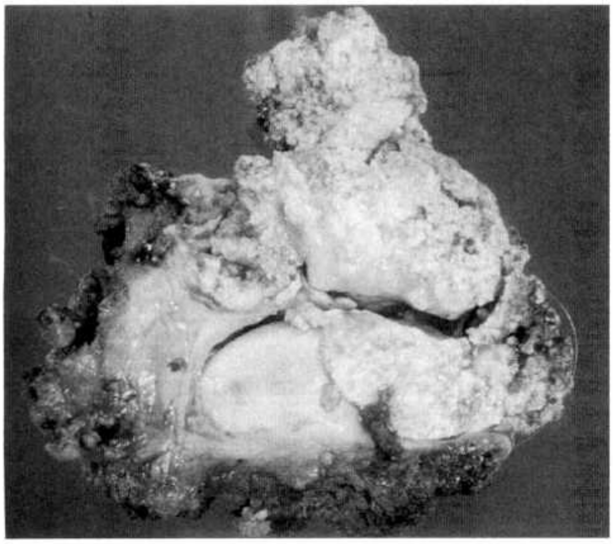

Fig 3. Cut surface of excised mass revealing white, chalky appearance.

Review of the reported cases reveals that disease involving the TMJ presents most commonly with 1 or more of the following: pain (57.9%), a swollen joint (42.1%), and trismus (36.8%). Two cases, 1 of which was ours, presented with minimal clinical symptoms. This was most likely due to lack of involvement of the mandibular condyle (Table 2[5,8-20]). Radiographically, the disease usually presents as a calcified mass involving the condyle or the joint space and degenerative changes of the condylar head.

Calcium pyrophosphate dihydrate deposition disease of the TMJ is a disease of older individuals, with the average age at the time of onset being 58.3 years, with a range from 41 to 80. There appears to be a female preponderance, with a 3:1 female-to-male ratio noted. Several systemic diseases have been associated with CPDD, including hyperparathyroidism, hypothyroidism, hyperphosphatemia, iron storage disorders, and rheumatoid arthritis.[6,21-25] Gout, which was present in our patient, coexists in only 5% of patients.[26]

The diagnosis is made by identifying the calcium pyrophosphate crystals in the joint and noting the usual linear punctate calcifications radiographically. The diagnosis is often a difficult one preoperatively, secondary to the ability of this disease to mimic other

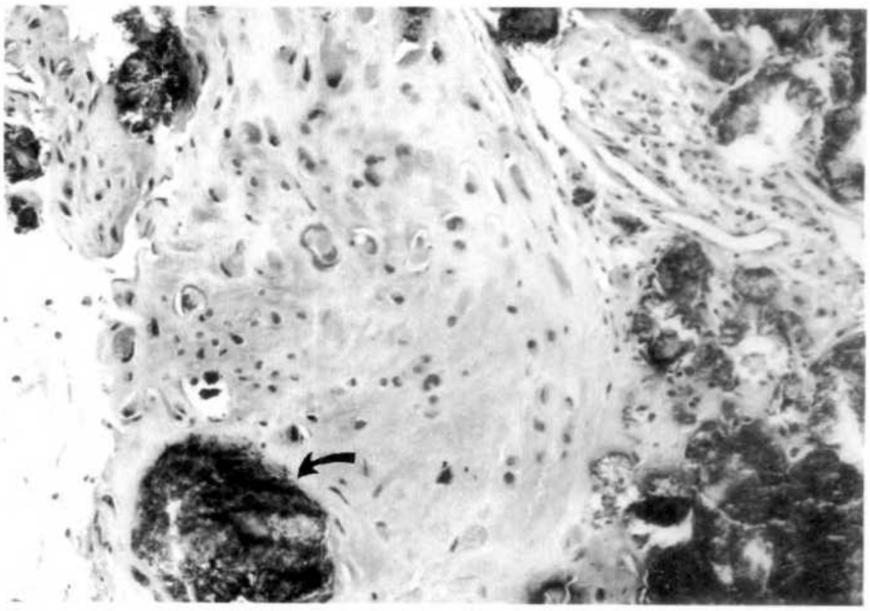

Fig 4. High-power view demonstrating area of chondroid metaplasia surrounded by islands of polarizable crystalline material consistent with calcium pyrophosphate (arrow).

disease processes, especially chondrosarcoma. Ishida et al[5] reported initial diagnoses of malignant carcinoma in 13 of 29 cases involving multiple different sites, in which the ultimate diagnosis of CPDD was made. Twenty-one percent of the 19 cases involving the TMJ were misdiagnosed as chondrosarcoma or chondroblastoma. In our case, the initial diagnosis by fine needle aspiration biopsy was low-grade chondrosarcoma, which significantly affected our management. Misdiagnosis as chondrosarcoma is particularly easy when decalcified sections are used, as the calcium pyrophosphate crystals are lost.[27,28] In addition, frozen sections possess more crystals than formalin-fixed sections and should be obtained, if possible, to help make a diagnosis.[17]

TABLE 1. REPORTED CASES OF CALCIUM PYROPHOSPHATE DIHYDRATE DEPOSITION DISEASE INVOLVING TEMPOROMANDIBULAR JOINT

Authors	*Year*	*No. of Cases*
Pritzker et al[8]	1976	1
deVos et al[9]	1981	1
Good and Upton[10]	1982	1
Zemplenyi and Calcaterra[11]	1985	1
Kamatani et al[12]	1987	1
Gross et al[13]	1987	1
Mogi et al[14]	1987	1
Hutton et al[15]	1987	3
Lambert et al[16]	1990	1
Magno et al[17]	1992	1
Dijkgraff et al1[18]	1992	1
Pynn et al[19]	1995	1
Chuong and Piper[20]	1995	1
Ishida et al[5]	1995	3
This study	1998	1

Histologically, CPDD presents with nodular, gritty granulation tissue within the joint space and erosion of the fibrocartilage. Microscopic evaluation often reveals foreign body giant cell reaction and associated chalky deposits, which should alert one to look for the presence of weakly birefringent rhomboid crystals when viewed with polarized light. Pressure erosion of the involved bone is usually seen. Chondroid metaplasia is often seen in association with the crystals and, if seen predominately in a biopsy, can lead to misdiagnosis. Because of its local destructiveness, vital structures can be involved. Several cases of cervical radiculomyelopathy were reported from CPDD involving the spine.[5] The exact growth rate of the tophaceous deposits is unknown. In our case, the lack of patient symptoms despite the massive bony destruction suggests a slowly evolving process.

The cause of CPDD is not completely understood. Elevated levels of inorganic phosphate are found within joint spaces without associated elevated serum levels. An abnormal cartilage matrix combined with these elevated phosphate levels may lead to

TABLE 2. SYMPTOMS OF CASES INVOLVING TEMPOROMANDIBULAR JOINT

	Cases	
Presenting Symptoms	*No.*	*%*
Pain	11	57.9
Swelling	8	42.1
Trismus	7	36.8
Abnormal occlusion	4	21.0
Hearing loss	3	15.8
No symptoms	2	10.5

Information from references 5 and 8-20, and present study.

crystal formation.[1,6] Crystal shedding from the cartilage surface may also be secondary to damage to the cartilage from trauma or chronic inflammation.[20]

Surgical treatment of tophaceous pseudogout is most appropriate when only 1 joint is involved, even though recurrence can occur even after complete excision. A literature review revealed 3 cases of recurrence in the TMJ an average of 2 years after excision. One patient had 2 recurrences.[5,18] However disease in other locations has been noted to recur up to 20 years after the initial resection.[5] In our case, with the apparent lack of clinical symptoms and advanced age of the patient, one could have made the argument for observation without aggressive resection. Preoperative error in diagnosis resulted in the patient's receiving an overly aggressive surgical procedure.

In conclusion, tophaceous pseudogout is a rare condition that may mimic many others, particularly chondrosarcoma. We present a unique case of tophaceous pseudogout that presented with marked destruction of the temporal bone. One should be aware of this entity when evaluating diseases of the temporal bone and TMJ.

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SUCCESSFUL PREVENTION OF RETROCOCHLEAR HEARING LOSS IN MURINE EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS WITH T CELL RECEPTOR Vβ8–SPECIFIC ANTIBODY

MIKIO SUZUKI, MD
OTSU, JAPAN
MARC S. KRUG, PHD
MEMPHIS, TENNESSEE

KUANG-CHUAN CHENG, PHD
MEMPHIS, TENNESSEE
TAI-JUNE YOO, MD, PHD
MEMPHIS, TENNESSEE

Experimental allergic encephalomyelitis is an animal model of a T cell–mediated autoimmune disease — for example, multiple sclerosis. We demonstrated that mice with experimental allergic encephalomyelitis developed retrocochlear hearing loss, and that the lesion of the auditory pathway might be related to T cell receptor Vß8–expressing T cells. To investigate whether anti-Vß8 antibody could prevent hearing loss, we carried out brain stem auditory evoked potential testing, histologic examinations, and flow cytometry in antibody-treated and control myelin basic protein–immunized B10.PL mice. The antibody was administered just before immunization of myelin basic protein. The disease incidence and severity were significantly reduced in the mice injected with the antibody. The results of brain stem auditory evoked potential testing, histologic examinations, and flow cytometry indicated that the depletion of Vß8-expressing T cells brings the prevention of hearing loss, as well as prevention of other neurologic deficits. The development of T cell receptor–specific antibody therapy might help treat retrocochlear hearing loss in multiple sclerosis.

KEY WORDS — anti–T cell receptor antibody, demyelination, experimental allergic encephalomyelitis, mouse, multiple sclerosis, retrocochlear hearing loss.

INTRODUCTION

Multiple sclerosis (MS) is one of the most frequent demyelinating diseases, resulting in a wide range of clinical signs and symptoms.[1,2] The disease most often runs a relapsing-remitting course with transient neurologic deficits. It is characterized by multiple demyelinating lesions disseminated throughout the white matter of the entire central nervous system (CNS). Microscopically, the lesions are located in the perivascular white matter of the CNS and characterized by lymphocytes, plasma cells, and macrophages with consequent inflammation and demyelination of the neuron sheaths; this leads to neurologic deficits, including paralysis and death. The cause of MS still remains obscure, but it is generally accepted that an autoimmune response is involved in its pathogenesis, and that genetic as well as environmental factors contribute in developing disease.

While sensorineural hearing loss as the initial manifestation of MS is rare, auditory involvement in MS is not infrequent.[3-6] It has been reported that the auditory system is affected in approximately 60% of patients with MS.[5] Musiek et al[7] demonstrated that more than 40% of their patients with normal peripheral hearing presented with subjective complaints of hearing difficulties. Magnetic resonance imaging demonstrated abnormalities in the brain stem, including the cochlear nucleus and the eighth nerve root-entry zone, as the responsible lesions for auditory involvement.[6,8]

Experimental autoimmune encephalomyelitis (EAE) in mice is induced by injection of myelin or myelin components, for example, myelin basic protein (MBP), with adjuvant; the mice show uncontrolled shivering, neuromuscular deficits, and coordination difficulties.[2,9,10] As the clinical symptoms and pathologic changes in EAE are similar to those in patients with MS, EAE is considered to be an animal model of MS.

We reported that MBP-immunized mice eventually developed retrocochlear hearing loss, as well as other neurologic deficits.[11] We found inflammatory cell infiltration in the inferior colliculus, superior olive, cochlear nucleus, and proximal portion of the

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From the Department of Medicine, Division of Allergy and Immunology (all authors), and the Microbiology and Immunology and Neuroscience Programs (Yoo), University of Tennessee, Memphis, Tennessee, and the Biomedical Research Center, Korea Advanced Institute of Science and Technology, Taejon, Republic of Korea (Yoo). Dr Suzuki is currently in the Department of Otolaryngology, Shiga University of Medical Science, Otsu, Japan. This study was supported in part by the University of Tennessee Medical Group Allergy Supporting Fund and by National Institutes of Health grant DC00652-04A3. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Tennessee.

CORRESPONDENCE — Tai-June Yoo, MD, PhD, Dept of Medicine, Division of Allergy and Immunology, University of Tennessee, Memphis, 956 Court Ave, Room H300, Memphis, TN 38163.

cochlear nerve, but not in the cochlea; some of the neural invasive cells expressed the Vß8.1,8.2 T cell receptor (TCR). The results of histologic studies suggest that T cells expressing Vß8 TCR might be related to the onset of retrocochlear hearing loss in EAE.[11]

Most recent research in EAE focuses on TCR usage. The usage of TCR V genes of encephalitogenic T cell clones derived from B10.PL (H-2^U) mice immunized by MBP is found to be restricted to the Vß8.2 gene segment (79%) and Vß13 segment (21%).[12] In PL/J (H-2^U) and (PL/J X SJL) F1 (H-2^{UXS}), the usage of TCR is also dominantly restricted to the Vß8 gene element.[13] On the basis of these results, treatment with monoclonal antibody against Vß8 was employed successfully to prevent or ameliorate disease in EAE mice.[12-15]

The aim of the present study was to investigate whether the TCR-specific antibody therapy (Vß8 antibody) that has been successfully applied to EAE could be extended to the hearing impairment in EAE mice.

MATERIALS AND METHODS

Animals. B10.PL mice were used in this study. Both sexes of B10.PL mice were obtained from Jackson Laboratories (Bar Harbor, Me) and bred at the Memphis Veterans Administration Medical Center animal research facility. Animals were from 6 to 8 weeks old at the time of disease induction.

Antigen for Induction of EAE. Myelin basic protein was prepared from frozen rat brain as previously reported.[11] The MBP extracts were lyophilized, and the concentration of MBP was deduced from 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).

Preparation of T Cell Receptor Vβ8–Specific Antibody. Anti-TCR Vß8 monoclonal antibody was purified from the supernatant of the F23.1 B cell hybridoma clone. The antibody produced by F23.1 B cell hybridoma is specific for Vß8 chains.[12,13,16] The clone F23.1 was grown in RPMI medium 1640 supplemented with 10% fetal calf serum, 1 mmol/L sodium pyruvate, 100 µg/mL penicillin, 100 µg/mL streptomycin, 2 mmol/L glutamine, 5 × 10^{-5} mol/L ß-mercaptoethanol, 20 mmol/L Hepes (pH 7.4), and nonessential amino acids. RPMI medium 1640, fetal calf serum, sodium pyruvate, penicillin-streptomycin, glutamine, Hepes (pH 7.4), and nonessential amino acids were purchased from Irvine Scientific (Santa Ana, Calif). ß-Mercaptoethanol was from Sigma (St Louis, Mo). A total of 1.5 L of supernatant containing anti-Vß8 monoclonal antibody was purified through protein A sepharose CL-4B (Pharmacia Biotech AB, Uppsala, Sweden). According to the manufacturer's recommendations, the supernatant was passed through a protein A sepharose column equilibrated with 20 mmol/L phosphate buffer (pH 7.0) and followed by 0.1 mol/L glycine buffer at pH 3.0 as an elution buffer. The eluted immunoglobulin (Ig) G was neutralized by 1 mol/L Tris–hydrochloric acid (HCl) buffer (pH 9.0). The monoclonal antibody was dialyzed in phosphate buffer (pH 7.0) and lyophilized. The antibody concentration was determined by 12% SDS-PAGE. The specificity of purified anti-Vß8 monoclonal antibody was confirmed by staining of TCR Vß8 cells harvested in B10.PL mice.[12,13,16]

Induction of EAE. The EAE was induced as previously reported.[11] Briefly, the mice were given a subcutaneous injection of a 0.1-mL emulsion consisting of equal volumes of 10 mmol/L acetic acid containing 200 µg of MBP and complete Freund's adjuvant (CFA) containing killed *Mycobacterium tuberculosis* (1 mg/mL, Central Veterinary Laboratory, United Kingdom). Three hundred nanograms of *Bordetella pertussis* toxin (List Pharmaceuticals, Campbell, Calif) was given intravenously 24 and 72 hours later.

The following scale was utilized to grade the clinical status of the mice: 0, no clinical disease; 1, tail weakness; 2, paraparesis; 3, paraplegia; and 4, quadriplegia or moribundity.[11]

Treatment of EAE With Anti-Vβ8 Antibody. The treatment group were injected with 0.5 mL 0.01 mol/L phosphate buffered saline (pH 7.4; PBS) containing 0.5 mg of Vß8 antibody into the peritoneal cavity just before a subcutaneous injection of the mixture of MBP and CFA as above mentioned. Controls received 0.5 mL PBS with 0.5 mg of nonspecific IgG1 or no treatment.

Recording of BAEPs. Under the anesthesia of ketamine hydrochloride (85 mg/kg) and xylazine hydrochloride (17 mg/kg), the BAEPs were recorded by a Nicolet CA-1000 system (Nicolet Biomedical Instruments, Madison, Wis). Investigation of the BAEPs was made with the mouse in a prone position in a dark, electrically shielded box. The body temperature was kept stable at approximately 37°C with a heating pad.

Auditory stimuli were applied through an earphone (MDR-E515, Sony, Japan) that was placed 1 cm from the right ear canal. The response signals were recorded with subcutaneous needle electrodes. The active electrodes were placed in the vertex and in the right retrocochlear region, with the ground on the back.

The sound stimulus consisted of 100-microsecond clicks at a repetition rate of 11.4/s. The evoked auditory potentials were amplified $\times 10^4$, filtered with a band pass between 150 and 1,500 Hz, and averaged from a total of 500 click-evoked responses for the first 10-millisecond period following stimulation. The stimulus intensity (decibels peak equivalent sound pressure level; dB pe SPL) was calibrated with a sound level meter (model 800B, Larson Davis, North Provo, Utah). The hearing threshold was determined as the lowest intensity for a visible wave I.

The measurement of BAEPs was performed on 0, 7, 14, 18, and 21 days postimmunization. The interpeak latencies (IPLs) at the stimulation of 110 dB pe SPL and the hearing threshold were measured in each mouse.

Histologic Studies. The mice showing a clinical score over 3 or reaching 21 days postimmunization were painlessly sacrificed in a carbon dioxide chamber. The mice were irrigated after intracardiac catheterization with PBS and then with 10% buffered formaldehyde for the ordinary hematoxylin and eosin (H & E) staining or with 10 mmol/L sodium periodate–75 mmol/L lysine–2% paraformaldehyde in 37.5 mmol/L sodium phosphate buffer pH = 6.4 (PLP) solution for immunohistologic staining. The brain tissue and temporal bone were removed. For H & E staining, the tissues removed were immersed in 10% buffered formaldehyde for 48 hours. For immunohistologic studies, they were immersed in PLP solution for 12 hours at 4°C. The tympanic cavities were widely opened and their stapes were removed for fixation, and after fixation they were decalcified in Tris buffer containing 10% ethylenediaminetetraacetic acid (pH 7.2) at 4°C.

For H & E staining, these tissues were dehydrated and embedded in paraffin, and 6-mm sections obtained were stained with H & E.

For immunohistologic studies, after fixation with PLP solution, the brains and the decalcified temporal bones were rinsed with 0.01 mol/L of PBS containing 10% to 20% sucrose for 36 hours at 4°C, embedded in OCT compound (Miles Laboratories Inc, Elkhart, Ind), and immediately frozen. Frozen sections of 6 to 10 μm in thickness were obtained. After rehydrating and rinsing in cold PBS, the sections were treated with 10% normal goat serum in PBS to reduce the nonspecific reaction. To investigate the lymphocytes that invaded the brains and temporal bones, unlabeled hamster anti mouse CD3 antibody (PharMingen, San Diego, Calif) and biotin conjugated mouse anti mouse Vß8.1,8.2 TCR monoclonal antibody (PharMingen) were used as the primary antibody. Both antibodies, diluted to 1:50 in PBS containing 1% bovine serum albumin (BSA), were applied to the sections and incubated for 1 hour at room temperature. After rinsing with PBS, the sections that were treated with anti-mouse CD3 antibody were incubated with biotin-conjugated goat anti hamster IgG (PharMingen) for 2 hours at room temperature. The secondary antibody was diluted to 1:50 dilution in PBS containing 1% BSA. After rinsing with PBS, the sections were incubated with avidin-biotin horseradish peroxidase complexes (Vectastain Elite ABC Kit, Vector Laboratories Inc, Burlingame, Calif) for 1 hour at room temperature and rinsed sufficiently with PBS. The specimens were developed with 0.02% 3,3'-diaminobenzidine in 0.05 mol/L of Tris-HCl buffer (pH 7.6) with 0.005% hydrogen peroxide and 0.07% NiCl6H2O. At this step, 0.065% sodium azide was added to the reaction solution to block the endogenous peroxidase activity.[17] The sections were dehydrated, cleared in xylene, and mounted for light microscopic examination.

Controls for the specificity of anti-CD3 antibody were studied with PBS or nonimmune hamster serum instead of primary antibody. Controls for the specificity of anti-Vß8.1,8.2 antibody were studied with PBS instead of the antibody. Control studies showed no immunoreactivity.

Fluorescence Flow Cytometry. To elucidate how anti-mouse Vß8 monoclonal antibody depletes the Vß8-specific T cells in vivo, fluorescence flow cytometry was performed on the mice that were injected intraperitoneally with 0.5 mg of anti-Vß8 antibody at day 8, day 15, and day 30 after injection. To compare with the results obtained from the mice receiving 0.5 mg anti-Vß8 antibody, flow cytometric examinations were also performed on the mice that were injected with 0.5 mg of mouse IgG1 (Cappel, Durham, NC) on the day of injection, and on the mice in the MBP-immunized + anti-Vß8 treatment group at 30 days postimmunization (DPI).

The lymphocytes were harvested from lymph nodes of these mice. The T cells were purified over nylon wool. The cells were washed twice with washing buffer (PBS, 5% fetal calf serum, 0.25% BSA, and 0.02% sodium azide, pH 7.4). The cells were treated with 10 μg/mL mouse anti mouse Vß8.1,8.2 antibody (PharMingen) at 37°C for 20 minutes. The labeled lymphocytes were washed with washing buffer, and then treated with fluorescein isothiocyanate–conjugated goat anti mouse IgG antibody (Cappel) at 37°C for 20 minutes. The cells were washed with PBS (pH 7.4), resuspended, and subjected to flow cytometric analysis using a flow cytometer at the University of Tennessee (50H cytofluorograph; Ortho Diagnostics System Inc, Westwood, Mass).

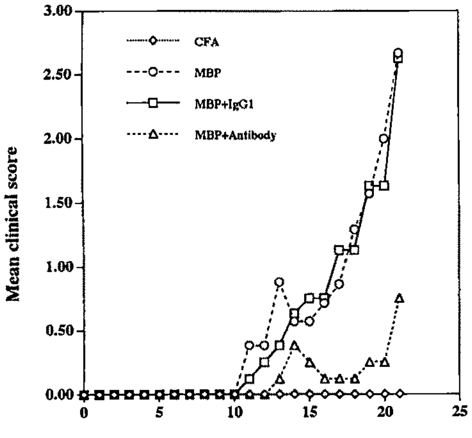

Fig 1. Clinical scores of mice. Value given is average score. In myelin basic protein (MBP)–immunized group, 1 mouse that developed experimental allergic encephalomyelitis (EAE) at 11 days postimmunization (DPI) was dead at 13 DPI. Clinical score after 14 DPI in this group did not contain data from this mouse. CFA — complete Freund's adjuvant–only control group; MBP — MBP-immunized group; MBP + IgG1 — MBP-immunized + nonspecific immunoglobulin G1 (IgG1) treatment group; MBP + antibody — MBP-immunized + anti-Vß8 treatment group.

Statistical Analysis. Statistical analysis was performed by Mann-Whitney U test or χ^2 test, as appropriate.

RESULTS

Experimental Design. The present study consisted of 4 groups: the CFA-only control group (4 males and 4 females) received a subcutaneous injection of emulsion of 10 mmol/L acetic acid and CFA; the MBP-immunized group (4 males and 4 females) received a subcutaneous injection of the equal-volume emulsion of CFA and 10 mmol/L acetic acid containing 200 μg of MBP; the MBP-immunized + nonspecific IgG1 treatment group (4 males and 4 females) received a subcutaneous injection of the emulsion of CFA and 10 mmol/L acetic acid containing 200 μg of MBP, and an intraperitoneal injection of 0.5 mg of nonspecific mouse IgG1 (Cappel); and the MBP-immunized + anti-Vß8 treatment group (4 males and 4 females) received a subcutaneous injection of the equal-volume emulsion of CFA and 10 mmol/L acetic acid containing 200 μg of MBP, and intraperitoneal injection of 0.5 mg of anti-Vß8 antibody (IgG1 isotype). All mice in each group were given intravenous injections of pertussis toxin 24 hours and 72 hours later.

FACS Analysis. The percentage of T cells expressing TCR Vß8.1,8.2 was 24% before injection of anti-Vß8 antibody. This percentage of Vß8.1,8.2-expressing T cells was consistent with previous reports.[12,13,16] The percentage of T cells expressing TCR Vß8.1,8.2 markedly decreased to 1.2% to 2.6% after injection of anti-Vß8 antibody. Nonspecific mouse IgG1 injection did not affect the population of T cells expressing TCR Vß8.1,8.2. At 30 days after the antibody injection, the depletion of T cells presenting TCR Vß8 was still observed. The T cell population expressing TCR Vß8 in the MBP-immunized + anti-Vß8 treatment group was 2.8% at 30 DPI (data not shown).

Clinical Observations. Clinical symptoms were found in all mice belonging to the MBP-immunized group and the MBP-immunized + nonspecific IgG1 treatment group (Fig 1). In the MBP-immunized group, 1 mouse that developed EAE at 11 DPI was dead at 13 DPI. Of the 8 mice in the MBP-immunized + anti-Vß8 treatment group, 2 mice (25%) developed EAE and showed clinical scores of 3. The incidence of diseased mice was significantly lower than those in the MBP-immunized and MBP-immunized + nonspecific IgG1 treatment groups (p < .01). No mouse developed EAE in the CFA-only group. The mean onset of the clinical signs was 16.0 ± 3.5 DPI in the MBP-immunized group, and 15.6 ± 3.7 DPI in the MBP-immunized + nonspecific IgG1 treatment group. The onsets of clinical symptoms of the 2 mice in the MBP-immunized + anti-Vß8 treatment group that developed EAE were 13 DPI and 14 DPI, respectively. Figure 1 shows the time course of the clinical signs. The time courses of the average clinical scores in the MBP-immunized and MBP-immunized + nonspecific IgG1 treatment groups were similar to each other. The time course of the average of clinical scores in the MBP-immunized + anti-Vß8 treatment group was located between the above two curves and that of the CFA-only control group. The average disease severities of the animals were 2.9 ± 0.4, 2.6 ± 0.7, and 0.8 ± 1.4 in the MBP-immunized group, the MBP-immunized + nonspecific IgG1 treatment group, and the MBP-immunized + anti-Vß8 treatment group, respectively. There were no significant differences between the MBP-immunized and MBP-immunized + nonspecific IgG1 treatment groups regarding the onset of clinical signs and the average disease severity. However, the average severity in the MBP-immunized + anti-Vß8 treatment group was significantly lower than those in the MBP-immunized and MBP-immunized + nonspecific IgG1 treatment groups (p < .05). Of the 8 mice in the MBP-immunized + anti-Vß8 treatment group, 3 mice that did not show clinical symptoms within 21 DPI were observed for a subsequent 2-month period; they did not show clinical symptoms during this observation period.

Comparison of Interpeak Latencies (Table 1). As wave V did not always appear, even in the CFA-only

TABLE 1. SUMMARY OF INTERPEAK LATENCY RESULTS AMONG FOUR GROUPS OF MICE

Group	*Interpeak Latency*	*Days Postimmunization* 0	7	14	18	21
CFA	I-II	0.72 ± 0.07	0.73 ± 0.04	0.72 ± 0.07	0.71 ± 0.10	0.72 ± 0.05
	I-III	1.55 ± 0.09	1.57 ± 0.04	1.55 ± 0.06	1.53 ± 0.05	1.55 ± 0.08
	I-IV	2.52 ± 0.09	2.51 ± 0.08	2.50 ± 0.06	2.53 ± 0.07	2.52 ± 0.08
MBP	I-II	0.71 ± 0.08	0.70 ± 0.07	0.75 ± 0.08	0.85 ± 0.15*	0.89 ± 0.11†
	I-III	1.52 ± 0.07	1.57 ± 0.08	1.62 ± 0.13	1.68 ± 0.08†	1.66 ± 0.06*
	I-IV	2.52 ± 0.05	2.56 ± 0.06	2.62 ± 0.09†	2.72 ± 0.13*	2.69 ± 0.08*
MBP + IgG1	I-II	0.71 ± 0.06	0.74 ± 0.05	0.77 ± 0.09	0.78 ± 0.08	0.84 ± 0.06*
	I-III	1.54 ± 0.06	1.54 ± 0.06	1.57 ± 0.08	1.63 ± 0.13	1.72 ± 0.06*
	I-IV	2.51 ± 0.03	2.53 ± 0.05	2.61 ± 0.05†	2.65 ± 0.09*	2.71 ± 0.06*
MBP + antibody	I-II	0.72 ± 0.06	0.74 ± 0.04	0.74 ± 0.06	0.75 ± 0.08	0.75 ± 0.10
	I-III	1.52 ± 0.04	1.53 ± 0.06	1.55 ± 0.06	1.55 ± 0.05	1.58 ± 0.13
	I-IV	2.51 ± 0.04	2.49 ± 0.06	2.52 ± 0.05	2.51 ± 0.06	2.51 ± 0.08

Data are milliseconds.

CFA — complete Freund's adjuvant–only control group; MBP — myelin basic protein (MBP)–immunized group; MBP + IgG1 — MBP-immunized + nonspecific immunoglobin G1 (IgG1) treatment group; MBP + antibody — MBP-immunized + anti-Vβ8 treatment group.

*p < .01.

†p < .05.

group, the analysis of IPL I-V was excluded from this study. The IPLs I-II, I-III, and I-IV did not change during the observation period in the CFA-only group. The brain stem auditory evoked potential (BAEP) results in each experimental group were compared to those of the CFA-only group. In the MBP-immunized group, the prolongation of IPL I-IV was found at 14 DPI and continued until 21 DPI. The prolongation of IPLs I-II and I-III was also found from 18 DPI. The prolongation of IPLs I-II and I-III in the MBP-immunized + nonspecific IgG1 treatment group was found at 21 DPI, and that of IPL I-IV started at 14 DPI. There were no significant differences in IPL delay between the MBP-immunized and the MBP-immunized + nonspecific IgG1 treatment groups. As a whole, the MBP-immunized + anti-Vß8 treatment group did not show significant IPL delays during the observation period, although the 2 individual mice affected by EAE developed slight IPL delays; 1 mouse had a slight delay at 21 days in IPL I-II only, and the other developed a delay in IPL I-IV at 18 DPI and in all IPLs at 21 DPI.

Hearing Threshold (Fig 2). The mean of the hearing threshold did not change during the observation period in the CFA-only group. The mean hearing thresholds of other groups were compared with that of the CFA-only group. In the MBP-immunized and

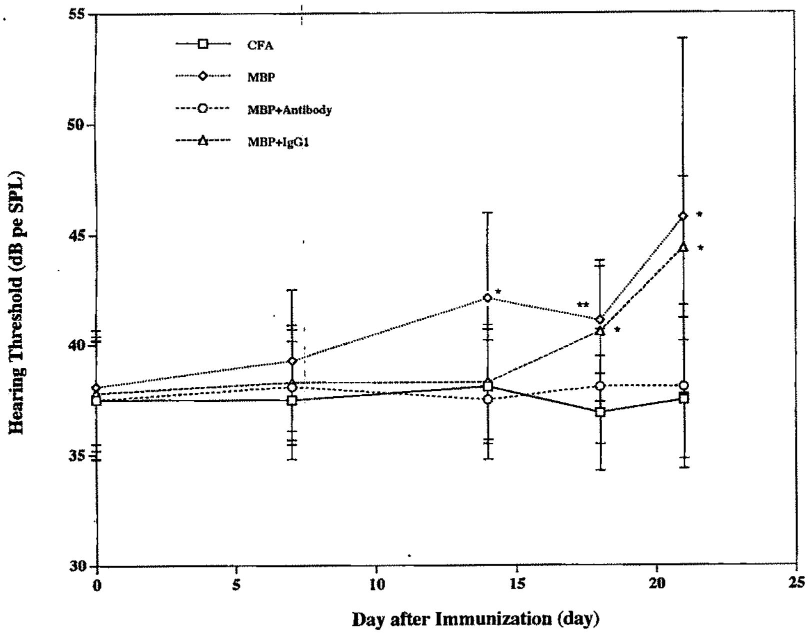

Fig 2. Brain stem auditory evoked potential (BAEP) results of control and immunized mice. Deterioration of hearing thresholds was observed in MBP-immunized and MBP-immunized + nonspecific IgG1 treatment groups. No significant changes were observed in CFA-immunized and MBP-immunized + anti-Vß8 treatment groups. * — p < .05; ** — p < .01.

TABLE 2. SUMMARY OF MORPHOLOGIC FINDINGS

	Immunohistologic Staining		
Group	*H & E*	*CD3*	*Vβ8*
CFA	–	–	–
MBP	+ to ++	+ to ++	+
MBP + IgG1	+ to ++	+ to ++	+
MBP + antibody			
With symptoms	+ to ++	+ to ++	–
Without symptoms	– to ±	–	–

Symbols –, ±, +, and ++ refer to degree of cell infiltration observed in cochlear nucleus. CFA — CFA-only control group; MBP — MBP-immunized group; MBP + IgG1 — MBP-immunized + nonspecific IgG1 treatment group; MBP + antibody — MBP-immunized + anti-Vβ8 treatment group.

MBP-immunized + nonspecific IgG1 treatment groups, the mean hearing thresholds deteriorated significantly starting at 14 DPI and 18 DPI, respectively. However, the net change of the hearing threshold remained small (approximately 8 dB pe SPL) in both groups even at 21 DPI. There were no statistically significant differences in the mean hearing thresholds between the CFA-only group and the MBP-immunized + anti-Vß8 treatment group during the observation period.

Histologic Studies. We examined H & E sections of 3 mice in the CFA-only group, 5 mice in the MBP-immunized group, 4 mice in the MBP-immunized + nonspecific IgG1 treatment group, and 4 mice in the MBP-immunized + anti-Vß8 treatment group. The results are summarized in Table 2. Inflammatory cell infiltration was observed in the cochlear nucleus in the MBP-immunized and the MBP-immunized + nonspecific IgG1 treatment groups (Fig 3A,B). Inflammatory cell infiltration was also observed in the inferior colliculus and cochlear nerve in these two groups of mice (Fig 4). There was no significant difference regarding cell infiltration between the two groups. This cell infiltration was observed especially around the vessels (perivascular cuffing) and leptomeningeal space. The infiltration into the parenchymal region was lower compared with those areas.

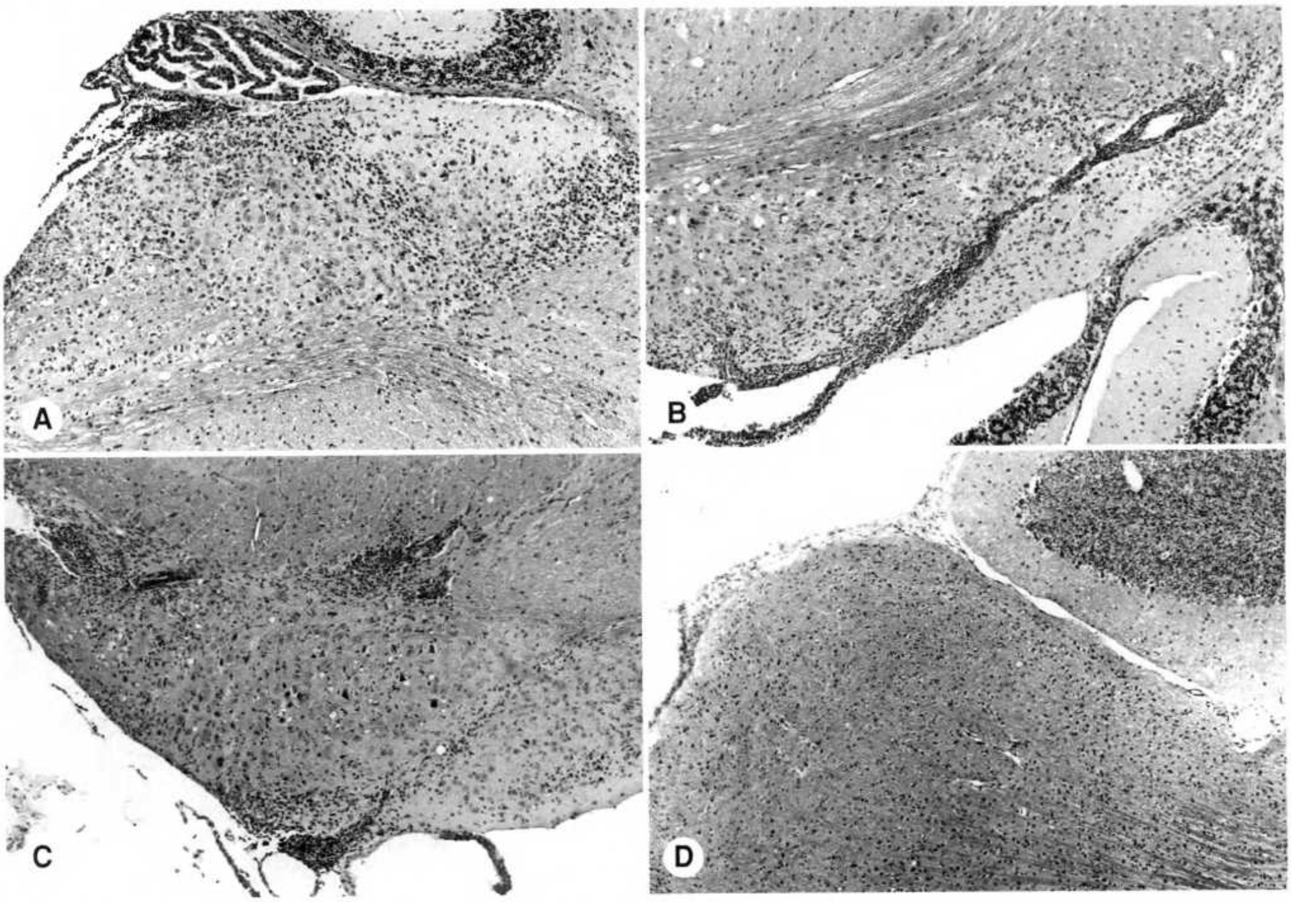

Fig 3. Inflammatory cell infiltration in cochlear nucleus of auditory tract (H & E, original ×100). A) This specimen was obtained from mouse in MBP-immunized group at 21 DPI. At this time, clinical score was 3. B) This specimen was obtained from mouse in MBP-immunized + nonspecific IgG1 treatment group at 21 DPI. At this time, clinical score was 3. C) This specimen was obtained from 1 of 2 diseased mice in MBP-immunized + anti-Vß8 treatment group at 21 DPI. At this time, clinical score was 3. Degree of cellular infiltration was similar to those in MBP-immunized and MBP-immunized + nonspecific IgG1 treatment groups. D) This specimen was obtained at 21 DPI from mouse in MBP-immunized + anti-Vß8 treatment group that did not show clinical symptoms. No pathologic changes were noted.

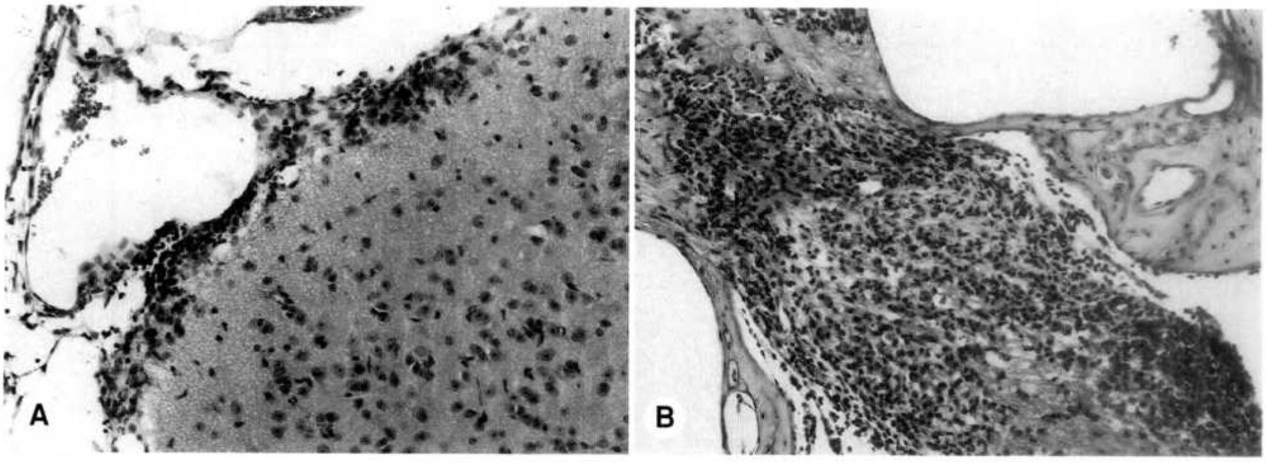

Fig 4. Inflammatory cell infiltration in auditory tract in MBP-immunized mouse (H & E, original ×200). A) Inferior colliculus. B) Cochlear nerve. Both specimens came from same mouse as in Fig 3A. No significant differences in H & E–stained sections were noted between MBP-immunized and MBP-immunized + nonspecific IgG1 treatment groups. Inflammatory cell infiltration was observed, especially around perivascular region and leptomeningeal space.

The cells infiltrating were mainly composed of lymphocytes and macrophages. There were no inflammatory changes in the cochlea. The cell infiltration into the cochlear nerve in the modiolus was also observed. However, the number of infiltrating cells was smaller in the spiral ganglion than in the inferior colliculus, cochlear nucleus, and cochlear nerve. These findings were consistent with our previous reports. The 2 mice that developed EAE in the MBP-immunized + anti-Vß8 treatment group also showed cell infiltration in those areas as well (Fig 3C). An absence of pathologic lesions was noted in histology sections of the other mice in the MBP-immunized + anti-Vß8 treatment group (Fig 3D). There were also no pathologic changes in the CFA-only group (data not shown).

Immunohistochemical examinations were performed on 2 CFA-only control mice, 3 mice in the MBP-immunized group, 2 mice in the MBP-immunized + nonspecific IgG1 treatment group, and 3 mice in the MBP-immunized + anti-Vß8 treatment group. As the most prominent cell infiltrations were found around the cochlear nucleus, the number of cells expressing CD3 or Vß8 was evaluated in this region. Cells presenting CD3 or Vß8.1,8.2 TCR were found in the cell infiltration of the cochlear nucleus in the MBP-immunized and MBP-immunized + nonspecific IgG1 treatment groups (Fig 5). One of the 2 affected mice in the MBP-immunized + anti-Vß8 treatment group was subjected to immunohistologic examination. There were numerous cells that were CD3-positive in the cell infiltration area (Fig 5C). On the other hand, Vß8.1,8.2-positive cells were not found (Fig 5D). The mice that did not present with EAE symptoms in the MBP-immunized + anti-Vß8 treatment group did not show either CD3- or Vß8-positive infiltrating mononuclear cells as did the CFA-only control group (Fig 5E,F).

DISCUSSION

Experimental allergic encephalomyelitis is an animal model of a T cell–mediated autoimmune disease and resembles MS in many respects.[2,9,10] The immunologic mechanisms underlying the disease process have been well characterized. Mouse EAE and human MS are both MHC class II–linked diseases, with H-2^u and H-2^s being the most susceptible haplotypes in mice.[18] The severity of clinical symptoms in EAE mice varies among the strains. In the present study, we used B10.PL mice that have H-2^u MHC background. The B10.PL mice in general develop a severe paralysis, most often resulting in the death of the animal. Encephalitogenic T cells from B10.PL mice respond to an MBP N-terminal peptide (NAc 1-9) in association with the I-A^u MHC molecule.[12-14]

Experimental allergic encephalomyelitis has served as a useful experiment model for investigating new therapeutic strategies in MS. Indeed, certain successful approaches for treatment of EAE are being attempted in MS.[19-21] A number of treatments of EAE have been reported, and Martin and McFarland[2] summarized them as the following: 1) treatment of EAE by blocking antigen presentation and adhesion of antigen-specific T cells, 2) treatment of EAE by altering T cell function, 3) modulation of EAE by lymphokines, and 4) modulation of EAE by different routes to administer myelin antigens. Anti-TCR monoclonal antibody therapy is a treatment of EAE that alters T cell function by targeting the encephalitogenic T cells. Monoclonal antibody against TCR Vß8.2 was applied successfully in the treatment of EAE in (PL/JXSJL)F1 and B10.PL

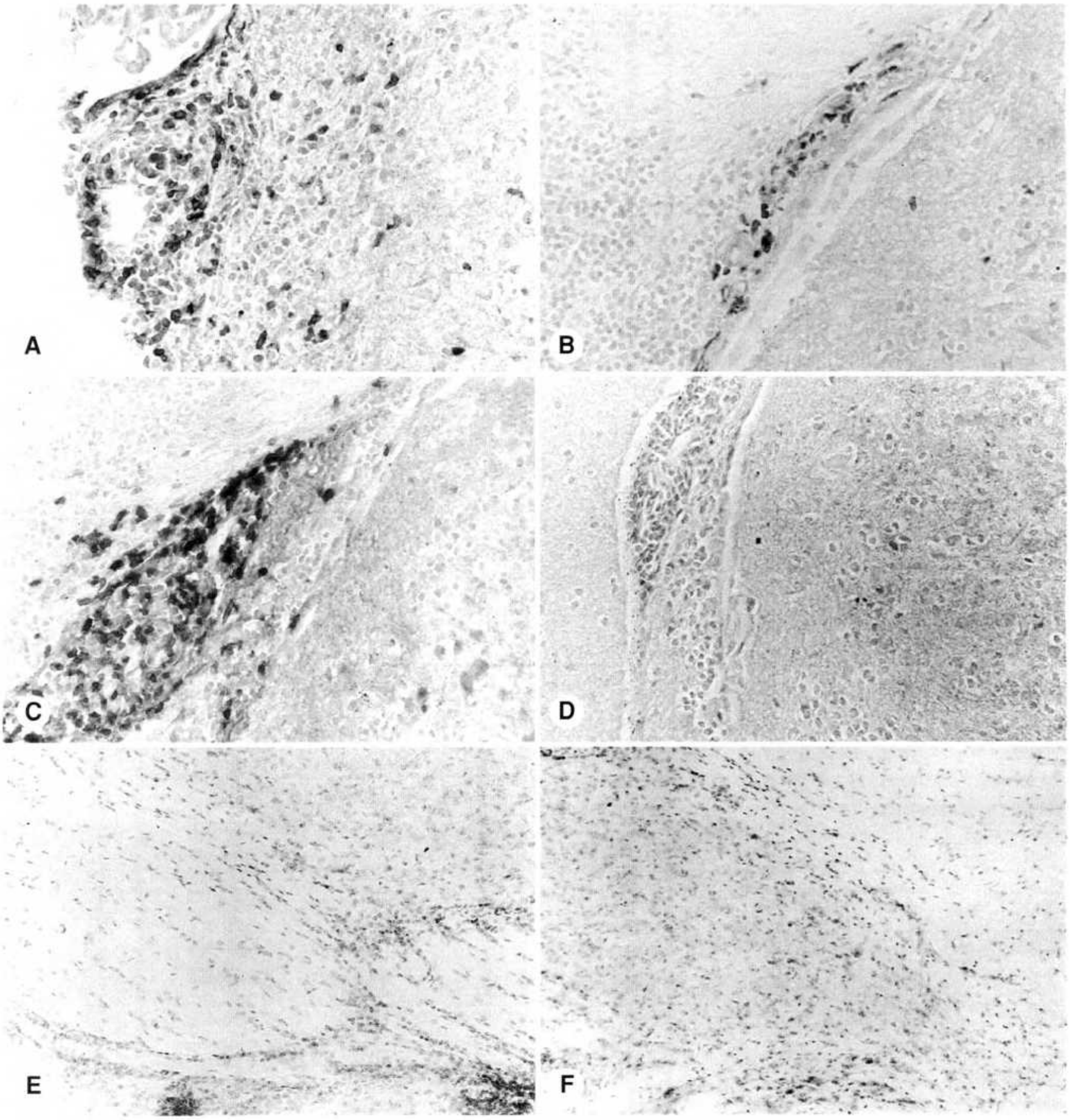

Fig 5. Immunohistologic demonstration of CD3- or Vß8.1,8.2-positive cells in cochlear nucleus. A) This specimen came from mouse in MBP-immunized group at 21 DPI (CD3 staining, original ×400). Majority of CD3-positive were observed in perivascular cuffs. There were some cells in parenchyma. B) This specimen came from same mouse as in A (Vß8.1,8.2 staining, original ×400). Majority of Vß8.1,8.2-positive cells were found in perivascular cuffs. C) This specimen was obtained at 21 DPI from mouse presenting clinical symptoms in MBP-immunized + anti-Vß8 treatment group (CD3 staining, original ×400). At this time, mouse had clinical score of 3. CD3-positive cells were observed in perivascular cuffing. D) This specimen was obtained from same mouse as in C (Vß8.1,8.2 staining, original ×400). No Vß8-positive cells were found in cell infiltration area. E) This specimen came from MBP-immunized + anti-Vß8 treatment group at 21 DPI (CD3 staining, original ×200). These mice did not show clinical symptoms. Specimens were counterstained with 1% neutral red after immunostaining. No positive CD3 cells were observed. F) This specimen was obtained from same mouse as in E (Vß8.1,8.2 staining, original ×200). No Vß8.1,8.2-positive cells were found.

mice.[12-15] Subsequently, vaccination with Vß8.2 peptide in Lewis rats reduced disease severity and speeded recovery.[22] This type of anti-Vß8 antibody therapy was also successfully employed in other autoimmune models.[23,24]

In the present study, the mice in the MBP-immunized and MBP-immunized + nonspecific IgG1 treatment groups developed severe paralysis in accordance with previous reports.[11,12,15] There were no significant differences in clinical symptoms, BAEP results, or histologic results between the MBP-immunized and MBP-immunized + nonspecific IgG1 treatment groups. These results indicate that administration of the mouse IgG1 isotype antibody to the mice immunized by MBP did not affect the EAE disease process. To put it another way, successful prevention of retrocochlear hearing loss in the MBP-immunized + anti-Vß8 treatment group was not simply due to the inoculation of mouse IgG1 isotype antibody. Significant delays of IPLs I-II, I-III, and I-IV and a deterioration of hearing threshold were observed in the MBP-immunized and MBP-immunized + nonspecific IgG1 treatment groups, just as in our previous report.[11] As significant inflammatory cell infiltration was found in the retrocochlear auditory pathway, but not in the cochlea, these abnormalities in BAEPs reflect retrocochlear hearing loss in the EAE mice.

In the MBP-immunized + anti-Vß8 treatment group, 2 of the 8 mice (25%) were afflicted with EAE, compared with 100% of the mice in the MBP-immunized and MBP-immunized + nonspecific IgG1 treatment groups. The average severity of disease symptoms of all animals in the MBP-immunized + anti-Vß8 treatment group was also markedly decreased, compared with the MBP-immunized and the MBP-immunized + nonspecific IgG1 treatment groups. An FACS analysis showed that depletion of Vß8.1,8.2-expressing T cells in the anti-Vß8–treated group continued for at least 30 days after anti-Vß8 antibody injection. Histologic examinations showed no pathologic lesions except for the 2 diseased mice in the MBP-immunized + anti-Vß8 treatment group. The results reveal that the depletion of Vß8-expressing T cells by anti-Vß8 antibody administration before immunization led to a significant reduction in the appearance of EAE. The mice in the MBP-immunized + anti-Vß8 treatment group that developed EAE showed a final clinical score of 3. The onsets of their clinical symptoms were at 13 and 14 DPI, which was not significantly different from those of the MBP-immunized and MBP-immunized + nonspecific IgG1 treatment groups. One possible explanation for the 2 cases of EAE in the Vß8-depleted mice is that the remaining Vß8-positive cells might have been sufficient to produce EAE. An FACS analysis showed that the mice in the MBP-immunized + anti-Vß8 treatment group still had some (2.8% at 30 DPI) Vß8-positive cells, although the number of positive cells was quite small. However, in our histologic examinations of 1 of the mice that developed EAE in the MBP-immunized + anti-Vß8 treatment group, CD3-positive cells but not Vß8-positive cells were found in the lesions. This observation strongly suggests that the EAE cases that escaped anti-Vß8 depletion were due to other MBP-reactive T cells. It has been reported that 79% of encephalitogenic T cells from B10.PL mice (H-2^u background) are expressing the TCR Vß8.2.[12] A minor population of those T cells expresses Vß13 (17%) or Vß4 (13%) in diseased B10.PL or PL/J mice.[12,13] Zaller et al[15] reported that injection of both anti-Vß13 and anti-Vß8.2 resulted in an enhanced reduction in EAE incidence when compared with injection of Vß8.2 alone. In this study, the failure of anti-Vß8 antibody to protect animals completely from EAE might be mainly due to the continued presence of MBP-reactive Vß13-expressing T cells.

In BAEP examinations, IPL delay and deterioration of hearing threshold were found in the MBP-immunized and MBP-immunized + nonspecific IgG1 treatment groups. In contrast, there were no significant differences in IPL and hearing threshold between the CFA-only control group and the MBP-immunized + anti-Vß8 treatment group. No pathologic lesions were noted in the MBP-immunized + anti-Vß8 treatment mice, except for the 2 mice displaying clinical symptoms. The histologic findings were consistent with the BAEP results. On FACS analysis, the depletion of Vß8-expressing T cells occurring in the MBP-immunized + anti-Vß8 treatment group was related to amelioration of the hearing impairment when the antibody was administered before induction of EAE.

It has been reported that encephalitogenic T cell clones derived from lymph nodes express Vß8.2 in rats as well as in mice.[12-15] In the rat EAE model, T cells expressing Vß8.2 TCR infiltrated the CNS at a very early stage of EAE and became the predominant population of infiltrating T cells.[25] Similar restriction of T cell usage was found in rats after infusion of an encephalitogenic T cell line.[26] In the mouse model, Vß8 cells were observed in the CNS starting 10 days after immunization, although multiple other Vß genes were also found.[27] The effect of anti-Vß8 antibody on EAE in this study suggests that T cells expressing Vß8 might have a key role in the appearance of hearing loss and other neurologic deficits. We are now preparing to investigate TCR usage in the retrocochlear lesions of EAE mice.

It is possible that the in vivo elimination of T cell subsets that express particular V region subfamily members could lead to reduced immune responsiveness and a consequent increase in susceptibility to environmental pathogens. However, the mice in the MBP-immunized and MBP-immunized + nonspecific IgG1 treatment groups did not display signs or symptoms of infection. As a matter of fact, it has also been reported that inbred mouse strains that lack 50% of the known Vß genes (SQL, C57K, C57BR, and SWR) do not exhibit obvious immunodeficiencies.[28] Although treatment with a monoclonal antibody to CD4[29] was also able to ameliorate EAE in mice, antibodies with high specificity to disease-causing TCR are desirable; antibodies with broader specificities lead to the depletion of a larger percentage of non–MBP-specific T cells and the consequent problem of immunocompromise. The approach in this study specifically eliminates the T cell subsets involved in EAE with minimal effect on overall immunity.

In summary, this study reveals that the anti-TCR monoclonal antibody prevents retrocochlear hearing loss in EAE mice, as well as other neurologic deficits. It is of interest whether the anti-TCR antibody can lead to reversal of retrocochlear hearing loss in EAE mice. The experiment needs to be done. There is a diverse T cell receptor repertoire in MS, and TCR gene utilization differs from that of EAE.[2,30] The characterization of TCR V gene usage in humans and the development of TCR-specific antibody therapy would help treat retrocochlear hearing loss in MS patients.

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MIDDLE EAR MECHANICS

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EFFECT OF HEALED TYMPANIC MEMBRANE PERFORATIONS ON UMBO VELOCITY IN THE RAT

DOUGLAS C. BIGELOW, MD
PHILADELPHIA, PENNSYLVANIA

DAVID KAY, MD
BROOKLYN, NEW YORK

JAMES C. SAUNDERS, PHD
PHILADELPHIA, PENNSYLVANIA

Heterodyne laser interferometry was used to measure tympanic membrane (TM) velocity at the umbo during acoustic stimulation in 2 groups of rats. The control group had a normal TM, while in the other group the TM was perforated and then allowed to heal for at least 28 days. Umbo velocity functions for constant sound pressure level stimuli were obtained for test tones between 0.3 and 40.0 kHz in each animal. The results revealed that velocity was the same in the control and healed TMs below 3.0 kHz. Above 5.0 kHz, the velocity response in the healed ear was between 3 and 12 dB smaller than in the control ears. Histologic evaluation of the healed perforation revealed a thick fibrous reaction between the epidermal and lamina propria layers. The results indicated that the added mass of the scar tissue changed the middle- and high-frequency TM responses.

KEY WORDS — laser interferometry, perforation, rat, tympanic membrane, umbo velocity.

INTRODUCTION

Perforations of the tympanic membrane (TM) are a common occurrence, often as a sequela of otitis media, barotrauma, or lacerating trauma including iatrogenic myringotomy.[1] It is known that nonhealed perforations of the TM in humans result in a low-frequency hearing loss.[1-3] While some perforations become chronic, the vast majority heal without gross evidence of hearing loss.[1]

There is a considerable literature describing the process of TM healing in both laboratory animals and humans. The rat TM achieves closure of rather large posterior perforations within 9 to 11 days.[4] Similar observations have also been made by others in the rat.[5,6] The process of repair begins with the rapid re-formation of the epithelial layer.[4,7-9] The epithelial layer apparently provides the substrate for regrowth of the lamina propria or middle layer. Most frequently, at least in the animal work, the repaired middle layer is much thicker than the normal lamina propria. The outer epithelial and inner respiratory layers, however, appear to return to normal thickness. In addition, the collagen organization of the lamina propria, in the region of the healed lesion, lacks the circular or radial organization of the original TM.[4-6,9] Repair of the TM in humans is much slower than that seen in laboratory animals, and the resulting replacement membrane may just as likely be very thin, appear normal, or be much thicker.[10,11] These differences in the profile of repair relate to the way in which the layers of the human TM regenerate.[10]

The functional capacity of the TM following the repair of a perforation is not well understood. The animal studies that examine sound transmission after the TM has healed have used indirect measures of middle ear function, and this makes it difficult to unambiguously determine the degree of functional recovery.[12-14] Similarly, human studies in general do not examine TM function at frequencies much above 6 to 8 kHz.

The observation of a perforation scar that exhibits a thickened TM whose lamina propria lacks the circular and radial collagen fiber organization suggests that the mechanical properties of the TM have changed. The present investigation employed laser interferometry to measure pars tensa velocity at the umbo tip in adult rats whose TM was perforated and then allowed to fully heal. It was hoped that these measures would provide a direct indication of the degree to which TM function returns following a perforation. The healed TMs were also examined histologically to determine the structural changes in the membrane from this trauma.

MATERIALS AND METHODS

Subjects and Groups. Results from 13 Long-Evans

From the Department of Otorhinolaryngology–Head and Neck Surgery, University of Pennsylvania, Philadelphia, Pennsylvania. This investigation was supported in part by an award from the National Institutes of Health/National Institute on Deafness and Other Communication Disorders to Dr Saunders (DC-00530). This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Pennsylvania.

CORRESPONDENCE — Douglas C. Bigelow, MD, Dept of Otorhinolaryngology–Head and Neck Surgery, 5 Ravdin — ORL, 3400 Spruce St, Philadelphia, PA 19104.

rats 56 to 74 days old are reported here. These animals were divided into 2 groups, 1 serving as unperforated control subjects and the other as the perforated group. Each animal was anesthetized (0.4 mg/kg sodium pentobarbital), and the posterior half of the TM was visualized through an operating microscope by manually retracting the pinna. A 27-gauge needle held securely in a hemostat was used to puncture the TM.[13] The sharp beveled edge of the needle was swept over the inner edge of the puncture to expand the size of the perforation. This procedure produced little if any bleeding at the perimeter of the wound.[13,15] The animal was then returned to the animal quarters and allowed to recover for a minimum of 27 days. At different intervals during this recovery period, all 5 of the perforated rats were again anesthetized and the condition of their TM was evaluated.

Surgical Preparation. At least 28 days after the perforation, both control and experimental animals were tested. Testing began by anesthetizing the animal with an intraperitoneal injection of 50% urethane (ethyl carbamate) solution. A tracheotomy was performed, and the external auditory canal and pinna were retracted to expose the posterior aspect of the TM. The skull was then secured in a head holder. The terminal zone of the external meatus[16] was resected to the outer perimeter of the tympanic ring to reveal the entire surface of the TM. Care was taken to avoid blood or instrument contact with the membrane, and to preserve intact and undamaged the annulus of the tympanic ring. The tissue over the posterior-lateral wall of the bulla was then exposed, and a small hole was made in the bulla at this location. The underlying endothelium on this hole was then pierced. A length of polyethylene tubing (30.0 mm long, 0.3 mm inside diameter) was inserted through the hole and into the bulla chamber. This tubing served to equalize the middle ear and atmospheric pressures.[17] The animal was moved to a sound-attenuated booth. Core temperature was maintained at 37°C with a rectal probe and a thermostatically controlled heating pad.

Sound Stimulation and Calibration. A frequency synthesizer (Audio Precision Inc, model System One) produced 122 test tones logarithmically spaced between 1.0 and 40.0 kHz (each lasting 0.75 second). These tones were presented free-field, and 2 speakers were used to cover the frequency range. An overlap region (5.0 to 10.0 kHz) between speakers was used to ascertain the accuracy of our calibrations. Sound pressure level (SPL) was measured through a probe tube (0.9 mm diameter, 37.0 mm long) microphone (Brüel and Kjaer, Inc, model 4134). The tip of the probe tube was positioned at the posterior edge of the TM, and the microphone response, expressed as decibels relative to 20 micropascals, was measured by the input section of the synthesizer. The synthesizer was programmed to adjust the stimulus level so that each test tone was presented at 100 dB SPL at the probe tube tip. Details of this calibration are presented elsewhere.[15,18]

Measurement of Umbo Velocity. A laser interferometer (Polytec Inc, model OFV-100) measured umbo velocity.[18-20] Reflected laser light from the acoustically stimulated TM was mixed with a reference beam, and the resulting interference pattern was converted to a voltage whose root mean square value was proportional to TM velocity. This voltage signal was connected to the input section of System One and converted to velocity in centimeters per second.

The laser beam was directed orthogonally toward the tip of the manubrium (the umbo). A glass bead, 10 to 30 μm in diameter, with a mass of about 5.0 ng was placed at the approximate center of the umbo tip to improve reflectivity. The bead was tightly coupled to the TM through the natural cerumen layer on the membrane surface, and its small mass did not appreciably increase the mass of the membrane.[15]

In several animals the laser beam was directed toward a portion of the skull immediately dorsal to the TM or onto the exposed bulla bone slightly lateral and posterior to the TM. The stimuli were then presented to the animal, at all frequencies, at 100 dB SPL, and the velocity of the skull at these points was measured. This provided an indication of the "noise floor" and could be used to assure us that the movements measured from the TM were not due to general vibrations of the skull.[15]

Procedure and Experimental Design. The high-frequency speaker was positioned above the head, and the laser beam was focused on the glass bead. The TM was stimulated at 100 dB SPL for 61 test frequencies between 5.0 and 40.0 kHz, and TM velocity was measured at each.

The low-frequency speaker was then positioned and velocity measured at another 61 frequencies between 1.0 and 10.0 kHz. The plot obtained by combining the results from both speakers provided an indication of the umbo frequency response. It took about 15 to 20 minutes to obtain data from both speakers, and a full protocol was obtained for the 8 control and 5 perforated animals.

A Polaroid picture of the healed perforation was taken after the TM was surgically exposed. A millimeter rule alongside the TM provided calibration for subsequent area measurements. The area of the pars

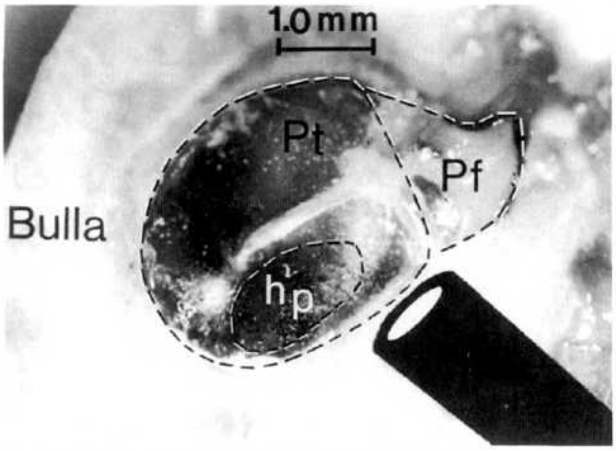

Fig 1. Photomicrograph showing tympanic membrane (TM) in adult rat. Glass bead was placed at tip of umbo, which is clearly visible through membrane. Location of perforation and its approximate size are indicated. Location of sound probe tube is also indicated. Pt — pars tensa, Pf — pars flaccida, hp — healed perforation. (Modified from Zimmer et al.[21])

tensa was then measured from the photograph by outlining the perimeter with the stylus of a digitizing tablet. A computer program (Jandel Inc, Sigma Scan) then calculated the area in square millimeters. The area of the healed perforation as revealed by the scar tissue was also measured. The healed area was compared to the total pars tensa area in order to determine the percentage of the pars tensa occupied by the healed perforation.

Histologic Preparation of TM. Once the velocity measurements from the healed perforation were obtained, the rat was painlessly sacrificed with a 2.5-mL intraperitoneal injection of a 50% urethane solution. The temporal bones were then harvested and fixed in 10% paraformaldehyde (pH 6.5) for 1 week.[21] The bones were then decalcified, embedded in paraffin, and cut in approximately 4.0-µm-thick sections. The TM was oriented so that the membrane was cut in such a way that the sections of interest passed through the approximate tip of the umbo and traversed the scar tissue formed by the healed perforation. The sections were stained with hematoxylin and Alcian blue. Control animals were prepared for histology in a similar manner.

Data Analysis. Umbo velocity was averaged over all animals, and standard errors of the mean were calculated for each frequency within a test condition. Averaged velocity functions were smoothed by calculating a running average response over successive 2- or 3-frequency intervals. Comparisons of TM velocity in the healed animals were made with the 20% open perforation velocity data previously reported.[15] In this way we could compare the response of the TM in control animals with a newly opened or a healed perforation.

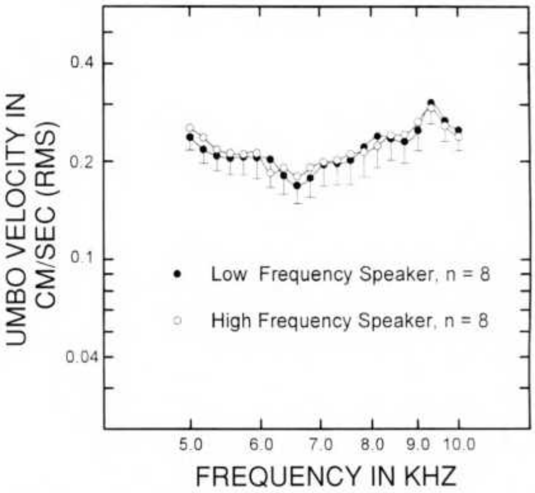

Fig 2. Average velocity response functions from control animals are shown between 5.0 and 10.0 kHz for low- and high-frequency speakers. Downward vertical bars indicate 1 standard error of mean for low-frequency speaker. This variability was typical of other speaker.

RESULTS

Control Conditions. Figure 1 shows a photomicrograph of the adult rat TM as viewed from the external meatus (photo modified from Zimmer et al[21]). The long arm of the manubrium can be clearly seen through the surface of the TM. The tip of the manubrium, referred to as the umbo, was the point at which laser velocity measures were obtained. The outlined area in the posterior quadrant of the membrane indicates the approximate location and size of the perforation. The location of the sound probe tube, just outside the tympanic ring, is indicated.

Figure 2 illustrates TM velocity in the nonperforated control ears for the overlapping frequencies from the low- and high-frequency speakers. The velocity values were relatively constant between 5.0 and 10.0 kHz, and more important, there were no overall velocity differences between the 2 speakers. The standard errors of the mean are only presented for the low-frequency speaker, but they were virtually the same for the other speaker. The similarity between the 2 conditions attests to the repeatability of our calibrations for the 2 acoustic drivers.

Umbo Response in Healed TM. A comparison between the TM velocity responses in the present group of control animals and a previously published control group is presented in Fig 3.[15] Both sets of control data have been smoothed by a 3-point running average. Previous published data from our laboratory on rat TM velocity have fewer data points over the same frequency range than our current control

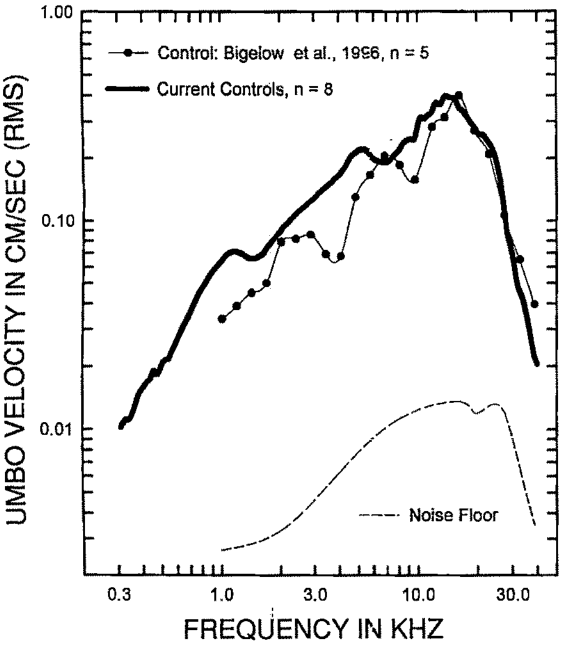

Fig 3. TM velocity function for group of present control animals is compared with control velocity functions reported elsewhere.[15] There was relatively little difference between 2 sets of data. Noise floor for vibration of skull adjacent to TM is also indicated.

data.[15] Nevertheless, the shapes of the velocity transfer function for the TM were very similar for the 2 sets of data. The low-frequency segment, between 1.0 and 15.0 to 18.0 kHz, rises at a rate of about 4 dB per octave for both functions. Similarly, above the response peak, the rate of decline in velocity is about 19 dB per octave for both sets of data. The slight differences in the magnitude of the low-frequency regions of the curve may be due to sound calibration. Between these 2 studies the sound probe tube was replaced with one having a slightly larger diameter and shorter length. Nevertheless, the similarity between these 2 sets of data allows us to make comparisons between the velocity functions for acute open perforations in the rat[15] and our present healed perforations. Figure 3 also shows the noise floor estimates as defined in Materials and Methods. The velocity of skull motion adjacent to the TM was between 25 and 30 dB below the motion of the TM at the umbo. Clearly, the signals we measured from the TM were not due to sound vibrations of the whole skull.

The Table presents data relating to the ages of the perforated animals reported in this study as well as the healing time, areas of the pars tensa and perforation, and, finally, the area of the healed perforation as a percentage of the total pars tensa area. As can be seen in the last column, the area of our perforations averaged about 16% of the total pars tensa area. It was difficult to accurately measure the area of the healed perforation, because the perimeter of the lesion was not sharply demarcated on the membrane. If anything, we suspect that these measures underestimate the true size of the lesion.

A comparison between TM velocity in the acutely perforated membrane and that in the healed membrane appears in Fig 4.[15] The data for a 20% perforation previously reported[15] are compared with the current results in the healed TM. Figure 4 illustrates the intact TM velocity functions for both studies. As can be seen, the principal consequence of a 20% hole in the posterior quadrant of the TM is a severe loss in low-frequency TM velocity (Fig 4A). The high-frequency velocity is nearly identical in control and perforated conditions above 4.0 to 5.0 kHz. Figure 4B shows that the low-frequency velocity functions for the healed TM were nearly identical up to 4.0 to 5.0 kHz. At frequencies above 5.0 kHz, however, the velocity functions were reduced in the healed TM when compared to the control results.

Figure 5 describes the gain of the TM velocity functions reported in Fig 4. These results were obtained by converting the ratio of the perforated data to the control data, at each frequency point, into a decibel value. As the results in Fig 5 show, there was a 22-dB reduction in velocity in the acutely perforated ear at 1.0 kHz, which declined to control levels by 4.0 to 5.0 kHz. In the healed ear above 4.0 to 5.0 kHz, there was a 3- to 12-dB loss in velocity. Statistical tests at selected frequencies between 6.0 and 40.0 kHz revealed that the velocity reduction in the healed TM was significantly lower than that of the control ear.

PERFORATION OF RAT TYMPANIC MEMBRANE

	Age at Perforation (d)	*Age at Experiment (d)*	*Perforation Healing Time (d)*	*Pars Tensa Area (mm²)*	*Perforation Area (mm²)*	*Perforation of Pars Tensa (%)*
	56	83	27	10.8	1.2	10.7
	61	93	32	8.5	1.9	22.1
	60	91	31	7.6	1.1	14.2
	61	91	30	7.9	1.5	18.8
	62	92	30	8.7	1.1	12.3
Average	60	90	30	8.7	1.3	15.6

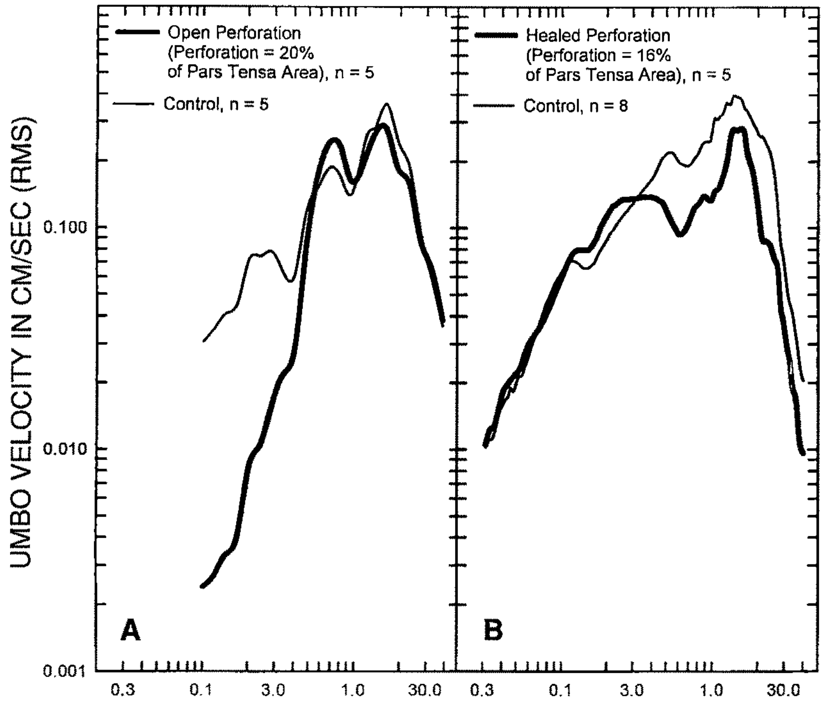

Fig 4. A) Comparison is made between control velocity functions and 20% acute perforations reported by Bigelow et al.[15] Note large low-frequency loss and similarity between 2 sets of data above 5.0 kHz. B) Similar comparison is made in healed TM. Note recovery of low-frequency response and emergence of new high-frequency velocity loss in healed animals.

Histologic Findings. When the TM was examined histologically in cross section (Fig 6), the healed area of the posterior TM appeared significantly thicker than the anterior portion of the TM, which was not traumatized. On closer examination, it appeared that the majority of this added tissue emanated from a thick fibrous reaction occurring between the epidermal layer, which was slightly thickened, and the relatively normal fibrous layer.

DISCUSSION

The validity of our results is supported by the consistency of the velocity responses in our control animals with those reported in other studies.[15,19] Moreover, the qualitative appearance of the umbo frequency response function in the intact TM condition exhibited characteristics similar to umbo velocity functions reported in other mammals such as the mouse, gerbil, and hamster.[18-20,22] These observations make us confident that the data reported here are a valid indication of middle ear sound transmission measured at the TM. In addition, the overlap of the high- and low-frequency speaker response curves gives us additional confidence in our calibration procedures.

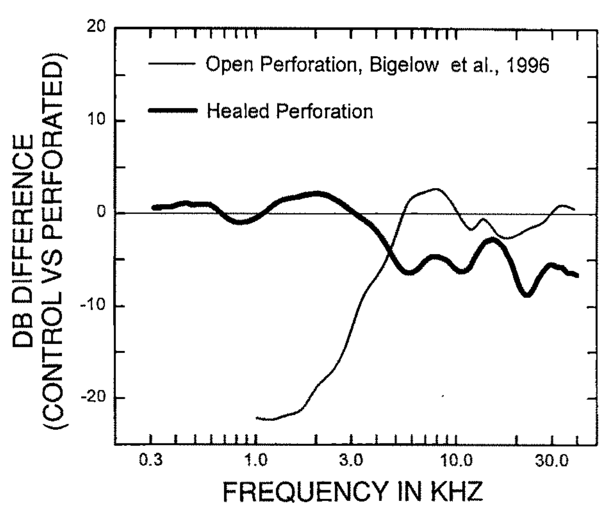

Fig 5. Relative decibel difference between control and perforated groups for results in Fig 4 is presented here. This more clearly shows low-frequency change in acutely perforated ears and high-frequency change in healed ears.

Tympanic membrane perforations are known to result in a low-frequency conductive hearing loss in humans,[1,3,23] and this has been further characterized in several animal studies.[12,15] It has been shown that the magnitude of the conductive loss increases and the range of frequencies affected widens as perforation size increases.[15] In fact, it has been demonstrated in the rat that umbo velocity drops to the level of the noise floor over most of the frequency range from 1.0 to 40.0 kHz as the size of the perforation encroaches upon 75% of the pars tensa.[15]

The majority of TM perforations, unless influenced by chronic infection, tend to heal on their own in humans, although at a relatively slow rate. This is true whatever the cause of the perforation, be it a burst injury from acute infection, barotrauma, a puncture injury, or perforation via a myringotomy knife.[10,11] In the laboratory rat the healing process is quite fast,[4-6] and in all of our animals examined between 7 and 10 days after perforation, closure was apparent. The healing process for a TM perfora-

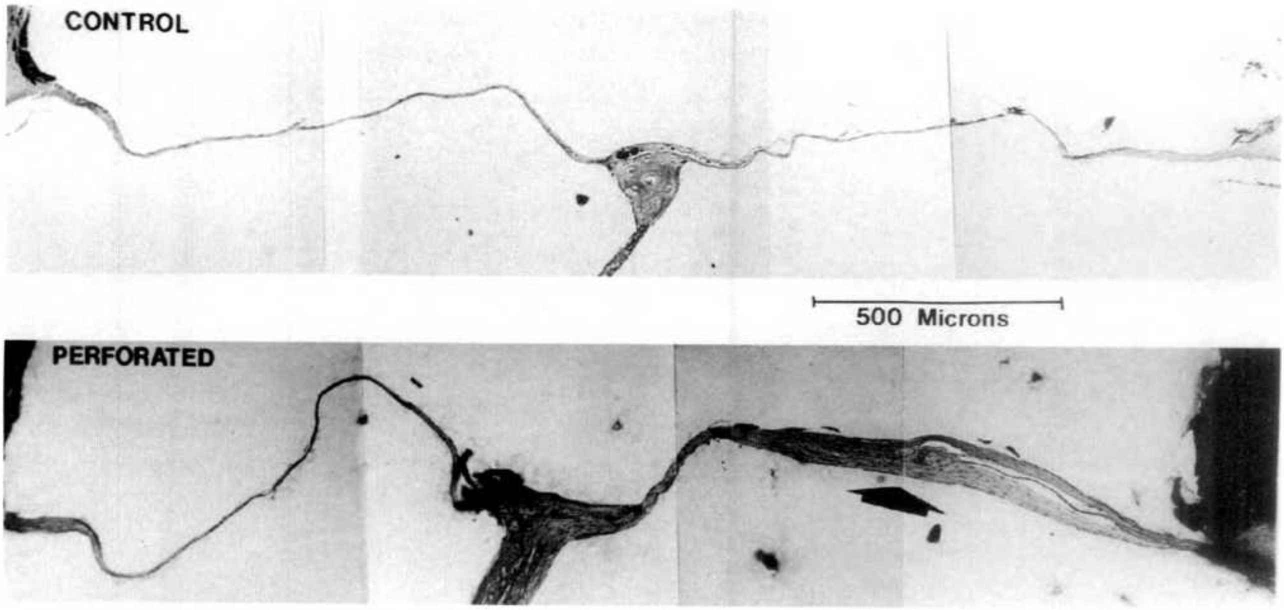

Fig 6. Upper panel illustrates section through normal TM, while lower panel is same view of TM with healed perforation. Note thickened membrane (arrow) in posterior region of TM.

tion has been characterized in previous animal studies.[4,7-9] Our histologic sections of healed TM perforations revealed similar findings, in that the scar tissue formation occurred mainly between the epidermal and fibrous layers and resulted in the addition of bulk to the TM in the area of the perforation.

Previous animal studies have examined the consequences of a healed perforation on sound transmission.[12-14] Indirect measures of middle ear function were employed, however, and this generated uncertainty about the actual degree of functional recovery. The present study directly measured the effect of healed TM perforations on umbo velocity. The method used in this study to make the perforation has been shown to cause no additional damage to the middle ear system, and creates a defect without actually removing any material from the TM.[15] The investigation, therefore, looks directly into the changes that occur in sound transmission from the natural healing process of a perforated TM.

The velocity curves we previously presented[15] showed a loss in low-frequency umbo velocity with a small, 20%, fresh nonhealed perforation when compared to a normal TM. When the perforation healed, the low-frequency response recovered completely. This recovery suggested that closure of the perforation restored the sound pressure gradient across the TM. In this way, the sound transmission properties of the TM at frequencies below 4.0 kHz were restored. However, a new loss in umbo velocity emerged for frequencies at 5.0 kHz and above. This change in sensitivity at these frequencies, however, was relatively small (between 3 and 12 dB).

The most likely cause for the alteration in high-frequency sound transmission in the healed TM would be the mass added to the membrane by the scar tissue over the lesion. Increasing the mass of the conductive apparatus has been well documented to alter the high-frequency response of the middle ear.[24]

Finally, the changes in the TM transfer function that we observed in this study were relatively small, and in the human patient are likely to be of little clinical significance. It is not clear if such a high-frequency loss in the human TM response would ever be identified. For one thing, most perforation recovery in humans results in a thinner TM.[10] In addition, the variability of standard threshold testing in human patients is at best about 5 to 10 dB with a reliable patient and experienced audiologist. Finally, conductive losses are difficult to reliably measure above 6 kHz and may well be below the level of detection by current audiometric instrumentation and procedures.

CONCLUSION

The data from this study demonstrate that the low-frequency alteration in sound transmission through the rat middle ear caused by a TM perforation is reversible on healing. The healing process, however, affects sound transmission in the middle to high frequencies between 5.0 and 40.0 kHz. It appears that this change in sensitivity is due to the added mass of scar tissue over the healed lesion. Increasing the mass of the conduction mechanism has been well documented to cause a loss in the high-frequency response. The changes in TM velocity on the healed membrane observed in this study were relatively small. If similar changes occur in the human patient after closure of a perforation, they are likely to be of little clinical significance.

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OTOLOGICAL MANIFESTATIONS OF CHARGE ASSOCIATION

INGEBORG DHOOGE, MD, PHD
GHENT, BELGIUM
MARK LEMMERLING, MD, PHD
GHENT, BELGIUM
MURIEL LAGACHE, MD
GHENT, BELGIUM
LIEVE STANDAERT, MD
BRUGES, BELGIUM
PAUL GOVAERT, MD, PHD
GHENT, BELGIUM
GEERT MORTIER, MD
GHENT, BELGIUM

The combined findings of abnormalities of the incus and stapes, absence of the oval window, absence of the stapedius muscle (with absent pyramidal eminence and tympanic sinus in most cases), and abnormalities of different parts of the inner ear (dysplastic cochlea, hypoplastic or dysplastic vestibule, and absent semicircular canals) are characteristic of the CHARGE association. Computed tomography and magnetic resonance imaging using 1-mm-thick contiguous slices proved useful in demonstrating the broad spectrum of temporal bone abnormalities in patients with the CHARGE association.

KEY WORDS — CHARGE association, computed tomography, temporal bone.

INTRODUCTION

In 1979, Hall[1] reported an association between choanal atresia and other congenital malformations. Pagon et al,[2] in 1981, suggested the acronym CHARGE to describe the nonrandom association of coloboma of the eye (C), heart anomaly (H), atresia of the choanae (A), retardation of growth and/or development (R), genital hypoplasia (G), and ear anomalies and/or deafness (E). The occurrence of other features has been noted with increased frequency.[3,4] Many children with the CHARGE association have been reported with facial paralysis, as well as feeding and swallowing difficulties. These are now considered to be important diagnostic components. The criteria for a definite diagnosis include ocular coloboma or choanal atresia and other anomalies constituting the acronym for a total of at least 4 defects.[2,5]

The cause of the CHARGE association remains unknown. Opitz[6] believes that the underlying events for an association are causally heterogeneous. Lubinsky[7] feels the CHARGE spectrum fails to meet the criteria for an association, but rather represents a variable syndrome with a more specific cause. Van Meter and Weaver[8] consider the CHARGE association an example of dysblastogenesis, due to abnormal migration of neural crest cells, deficiency of mesodermal formation, or abnormal interaction between mesoderm and neural crest cells. Most cases occur sporadically. Some familial cases of CHARGE association have been reported, probably due to a single gene mutation.[9,10] Different chromosomal abnormalities have been reported in cases of CHARGE association, suggesting that several genes may play a role in its pathogenesis.[11-14]

In focusing on ear anomalies, the "E" traditionally refers to the mixed hearing loss (conductive and sensorineural) noted in these patients and to the external ear malformations. Ear anomalies and/or hearing impairment are significant features in CHARGE association.[15,16] This report describes the external, middle, and inner ear malformations in 11 patients with CHARGE association on the basis of clinical examination, audiologic evaluation, and computed tomography (CT) and magnetic resonance imaging (MRI) findings in an attempt to delineate criteria suggestive for the existence of the association.

SUBJECTS AND METHODS

Eleven patients were reviewed. Inclusion into this report required that patients had ocular coloboma or choanal atresia and other anomalies constituting the acronym for a total of at least 4 defects.[2,5] One patient was included with only 3 major criteria but with presence of other anomalies associated with the CHARGE association (Table 1). An otological examination was performed by a skilled otoscopist. The external ears were all photographed and scored by 2 independent observers for any abnormal morphology. The audiologic evaluation of these patients was carried out with the most appropriate technique for the patient's age and developmental status. Conventional behavioral audiometry was attempted. Auditory brain stem responses (ABRs) were measured with alternating-polarity clicks of 100-microsecond duration presented through standard earphones. In most cases, anesthesia was required to perform ABR tests. If possible, hearing loss was later confirmed by free field audiometry using both tones and speech

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From the Departments of Otolaryngology (Dhooge, Lagache), Radiology (Lemmerling), and Medical Genetics and Pediatrics (Govaert, Mortier), University Hospital Ghent, Ghent, and Spermalie Institute, Bruges (Standaert), Belgium.

CORRESPONDENCE — Ingeborg Dhooge, MD, PhD, Dept of Otolaryngology, University Hospital Ghent, De Pintelaan 185, 9000 Ghent, Belgium.

TABLE 1. CLINICAL FINDINGS IN PATIENTS WITH CHARGE ASSOCIATION

	Patient No.										
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
Coloboma ("C")	+	+	–	+	+	+	–	–	+	+	–
Heart anomaly ("H")	+	–	+	+	–	–	+	+	+	+	+
Choanal atresia ("A")	+	–	+	–	–	–	–	+	–	–	–
Retarded growth ("R")	+	+	+	+	+	+	+	+	+	+	+
Hypogenitalism or cryptorchidism ("G")	+	+	+	+	–	–	–	+	–	+	+
External ear malformation ("E")	+	+	+	+	+	+	+	+	+	+	+
Hearing loss	+	+	+	+	+	+	+	+	+	+	+
Middle ear disease	+	+	+	+	+	–	+	–	–	+	+
Facial palsy	+	–	–	–	–	+	+	+	–	+	+
Feeding problems	+	–	–	–	+	+	+	–	+	+	–
Brain anomaly	–	+	–	–	+	+	+	+	–	–	–
Cleft lip or palate	+	–	–	–	–	+	–	–	+	–	–
Micrognathia or retrognathia	–	+	+	+	+	–	–	+	+	–	+
Skeletal anomaly	+	–	+	+	–	–	–	–	–	+	+
Mental retardation	+	–	+	+	–	–	?	?	?	+	–

stimuli. The signals were warbled tones, bands of noise, and monitored live speech. Threshold results were classified as indicating normal peripheral hearing (0 to 20 dB) or impaired hearing of a mild (20 to 40 dB), moderate (40 to 70 dB), severe (70 to 90 dB), or profound (>90 dB) degree.

Computed tomography scans of the temporal bones from 7 patients were included in the study. One of the patients underwent a high-resolution MRI examination of the temporal bones using 1-mm-thick 3DFT-CISS (3-dimensional Fourier transformation–constructive interference in steady state) weighted images.[17]

RESULTS

Seven male and 4 female patients were included. One patient died neonatally of severe cardiac malformation. Of the 10 remaining patients, the age at follow-up ranged from 19 months to 24 years. In 2 cases polyhydramnios was found during pregnancy. Both children had severe feeding and swallowing difficulties at birth. Findings on chromosomal analysis were normal in 10 patients. In 1 case we found an abnormal karyotype (mosaic 46XY/47XY13q–). One patient has a performance IQ of 77 (WISC-R). The other patients are mentally retarded.

Clinical Findings. Table 1 summarizes the clinical findings in the 11 patients. All patients had external ear malformations, hearing loss, and retarded growth. Coloboma of the eye also occurred very frequently (7/11). Unilateral peripheral facial palsy was present in 6 patients. All male patients had hypogenitalism or cryptorchidy. All children were considered hypotonic early in infancy. Most of the children had feeding and swallowing difficulties in early childhood. Of the brain anomalies, a large fourth ventricle was the most frequent finding. Skeletal anomalies included syndactyly and clinodactyly of the fifth finger

TABLE 2. ANALYSIS OF EXTERNAL EAR MORPHOLOGY

	Patient No.										
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
Short and wide ear*	+	–	+	+	+	+	+	+	+	+	+
Triangular concha*	+	–	+	+	±	±	+	+	+	+	+
Discontinuity of antihelix and antitragus	+	+	±	+	+	–	+	+	+	–	–
One crus missing	±	+	+	+	±	±	+	+	+	–	+
Prominent crura	–	–	+	+	±	–	–	±	–	–	+
Snipped-off portions of helical fold	–	–	±	–	+	–	+	±	+	–	–
Small or absent lobes*	+	+	±	+	+	+	+	+	+	+	+
Asymmetry between 2 ears	+	–	+	+	+	+	+	+	+	–	+
Preauricular tags	–	–	–	–	–	–	±	–	–	–	–
Low-set and/or posteriorly rotated pinnae*	+	–	±	+	+	+	+	±	+	+	+
Microtia	–	–	–	–	–	–	±	–	±	–	–

+ — Anomaly present in both ears; ± — anomaly present in 1 ear; – — anomaly absent.

*Most prevalent findings.

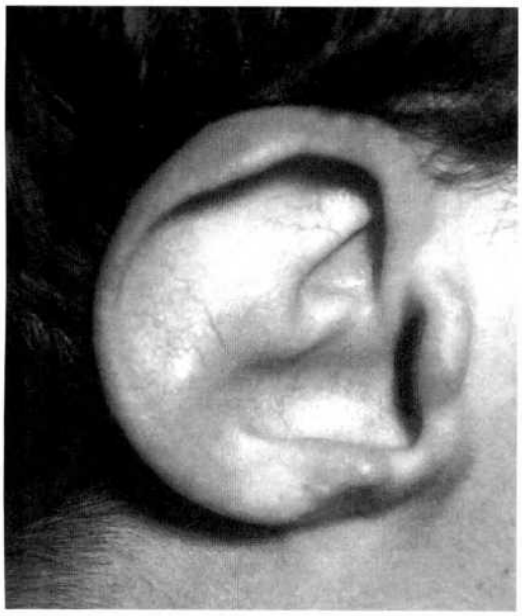

Fig 1. External ear malformations in patient with CHARGE association.

(5 patients).

External Ear Malformations. All patients had abnormal external ears. The external ears of 11 patients were scored according to a list of malformations found in the literature mainly described by Davenport et al.[18] The results are shown in Table 2. Most ears were short and wide, with a triangular concha (10/11; Fig 1). They were almost always low-set and posteriorly rotated. Other very frequent findings were small or absent lobes (11/11) and discontinuity between the antihelix and the antitragus (8/11). The crura were sometimes very prominent (5/11). One crus was often missing (10/11). Portions of the helical fold were sometimes missing (5/11). There were 2 cases of microtia. There were no cases of atresia or stenosis of the outer ear canal.

Middle and Inner Ear Anomalies. Recurrent or persistent middle ear disease (otitis media, tympanic membrane perforation) was reported in 7 patients. Computed tomography scans of the temporal bone showed that of the 14 ears of those 7 patients, all but 2 had an abnormal incus. The appearance of the stapes was always rated abnormal, ranging from hypoplastic to absent (Fig 2). Fixation of the ossicular chain was a consistent finding (Fig 2), with fixation of the head of the malleus to the anterior tympanic wall being most frequent. Opacification of the tympanic cavity was observed in all but 1 ear. All temporal bones showed absence of the stapedius muscle, with absence of the pyramidal eminence and tympanic sinus in 11 ears (Fig 2). In all instances the oval window was absent (Fig 2). The round window was present in all cases, but obliteration of its niche sometimes occurred due to soft tissue; in 6 ears of the 14, obliteration was due to osseous septa (Fig 2).

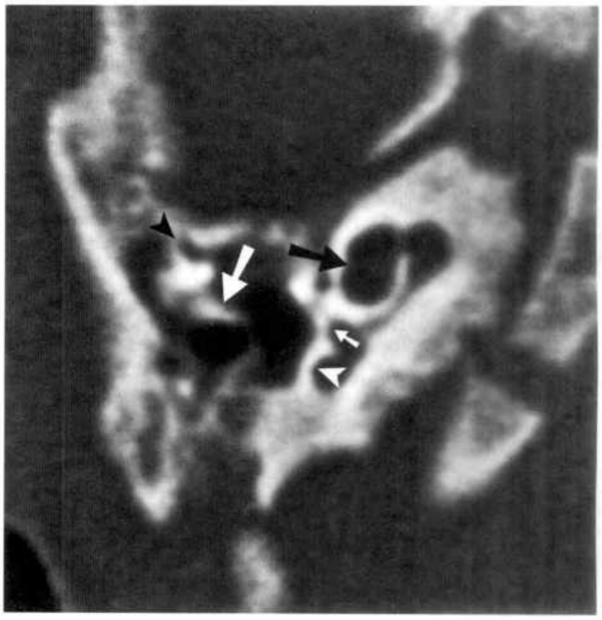

Fig 2. Axial 1-mm-thick computed tomography section through right mesotympanum in patient with CHARGE association shows opacification of hypoplastic middle ear cavity. Note fixation of malleus head to anterior tympanic wall (black arrowhead), malformation and malrotation of incus (large white arrow), and absence of stapes. Also note absence of oval window (small white arrow) and presence of round window niche with bony obliteration (white arrowhead). Tympanic sinus, pyramidal eminence, and stapedius muscle are absent. Interscalar septum between middle and apical turns of cochlea is missing, as well as normal cochlear modiolus (black arrow).

In all cases, abnormalities of the bony labyrinth were seen — of the cochlea as well as the vestibule and semicircular canals. Cochlear anomalies were seen in 12 of the 14 ears, ranging from subtle modiolar deficiencies to more severe partition defects (Mondini malformation; Fig 2). The vestibule was always present, but generally hypoplastic or dysplastic. All patients showed aplasia of the semicircular canals (Fig 2). In 8 ears, the course of the facial canal was difficult to trace, especially in the tympanic segment.

The MRI scan of 1 patient confirmed the hypoplastic aspect of the vestibule and absence of the semicircular canals. An abnormal interscalar septum at the cochlear apex was also demonstrated (Fig 3).

Hearing Loss. All patients had impaired hearing. Three of the 11 had a bilateral profound hearing loss, and 5 of the 11 had severe hearing loss. Three had a moderate loss in the best ear.

In 1 patient the typical wedge-shaped audiogram — flat air conduction thresholds with downward-

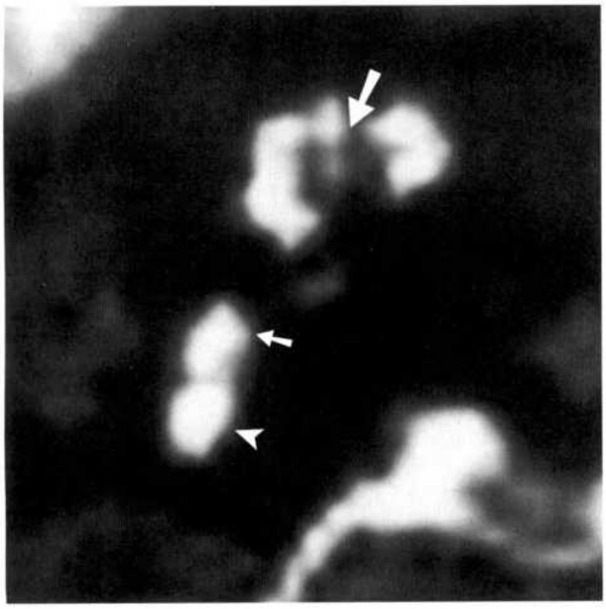

Fig 3. Axial 1-mm-thick 3DFT-CISS (3-dimensional Fourier transformation–constructive interference in steady state) weighted magnetic resonance image through inner ear in patient with CHARGE association. Note abnormal cochlear apex with lack of development of apical cochlear turn (large arrow). Vestibule is hypoplastic (small arrow), and stump of posterior semicircular canal is seen (arrowhead). Lateral semicircular canal is absent.

sloping bone conduction levels — was found (Fig 4). In 9 of the 11, the diagnosis of hearing loss was ascertained by ABR. Three patients were abnormally resistant to anesthetic gases. In 6 cases the hearing loss was later confirmed by free field audiometry with tones and speech delivered through speakers. In only 3 cases, conventional behavioral audiometry was possible, which showed a mixed hearing loss. In 1 of the patients with a mixed hearing loss, surgical exploration of the worse ear was attempted. An aberrant course of the facial nerve was found that ran over the oval window. Reconstruction of the ossicular chain was therefore impossible. In 8 of the 11 patients, a hearing aid was fitted unilaterally.

Equilibrium. For 6 patients there is detailed information on the neuromotor development during the first years of life. The following symptoms were striking and present to a greater or lesser degree in all 6 patients: hypotonia during the first years of life, poor head support, slower placing responses of the upper extremities, delay in sitting without support (never before the age of 1 year), delay in walking (not before the age of 2 years), and difficulties in transition from one position to another. Two older patients reported evident vestibular problems compatible with a bilateral areflexia of the labyrinth (problems with walking in the dark, problems with walking on uneven ground). None of the patients had vestibular testing.

DISCUSSION

Since the first description of the acronym CHARGE by Pagon et al[2] in 1981, many other features have been reported with increasing frequency and are considered equally important. Feeding and swallowing difficulties, brain anomalies, facial palsy, and skeletal anomalies (micrognathia or retrognathia, clinodactyly) were present in at least half the patients in our study. Although the patients differed in the spectrum of clinical features of the association, all suffered ear malformations with striking similarity in the 3 segments (outer ear, middle ear, and inner ear).

The first review of external ear malformations in CHARGE association was published by Davenport et al.[18] The results of our study confirm consistent findings: low-set and posteriorly rotated ears, short and wide pinnae with triangular conchae, small or absent lobes, and discontinuity between the antihe-

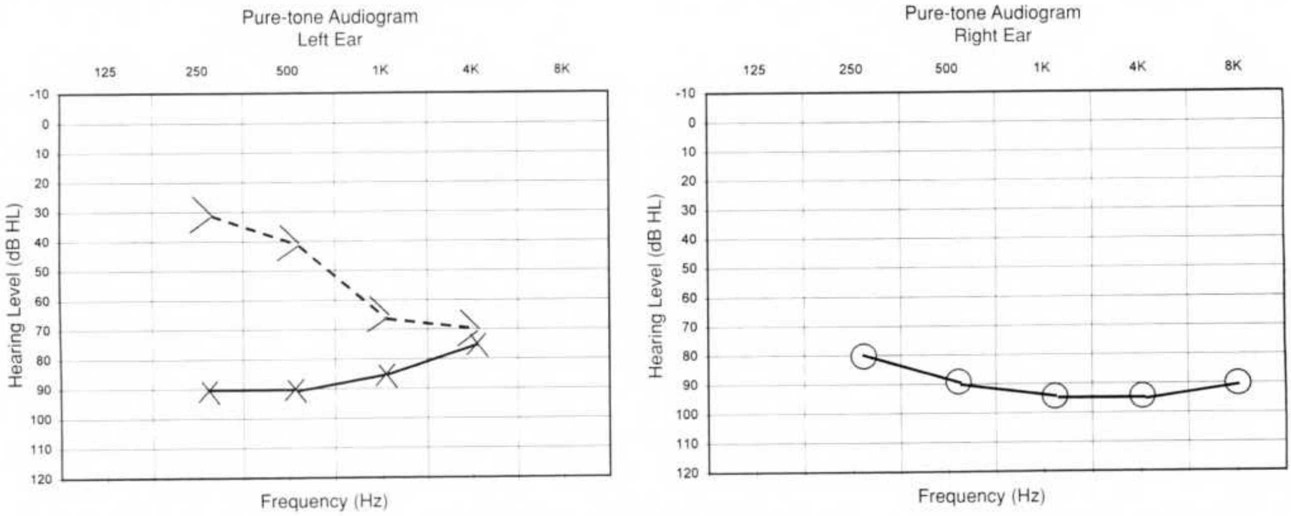

Fig 4. Typical wedge-shaped audiogram, ie, flat air conduction thresholds with downward-sloping bone conduction levels.

lix and antitragus. The external ear anomalies in CHARGE association are so distinctive that a presumptive diagnosis can sometimes be made on that basis alone.

In our study, as well as in the literature, there is mention of persistent middle ear disease. Due to the more intense medical follow-up, middle ear problems are probably more frequently reported in this group of patients. But there is often persistent middle ear effusion at an age when it is uncommon, possibly due to eustachian tube dysfunction from choanal atresia.[19] Middle ear problems can cause fluctuating hearing loss, making a correct evaluation of the hearing difficult. Early diagnosis and intervention (eg, tube placement) is important to optimize hearing.

The CT scans of the temporal bones showed very characteristic malformations of the middle and inner ear, some of which had not been mentioned in the literature. It is clear from this study that a large number of abnormalities of the middle and inner ear possibly contribute to the mixed hearing loss seen in patients with the CHARGE association: malformed or absent ossicles (incus and stapes), fixation of the ossicular chain to the wall of the tympanic cavity, opacification of the tympanic cavity, absence of the stapedius muscle, absence of the oval window, obliteration of the round window, and dysplasia of the cochlea.

In a report by Morgan et al,[16] the CT finding of a hypoplastic incus combined with absent semicircular canals is considered to be pathognomonic of the CHARGE association. Guyot et al[20] suggested that the inner ear abnormalities seen in the CHARGE association, consisting of an aplasia of the pars superior and a Mondini deformity of the pars inferior of the inner ear, represented a specific form of labyrinthine dysplasia, which they called CHARGE dysplasia of the temporal bone.

The findings in our present study, with absence of the semicircular canals in all cases, cochlear malformations in 86% of the cases, and incudal abnormalities in 86% of the cases — in particular of the long process of the incus — support this hypothesis. Two other interesting and consistent radiologic findings in our series, namely, the absence of both oval window and stapedius muscle, should be added to this combination. Support for the finding of an absence of the stapedius muscle on imaging can be found in the histopathology literature.[21] This finding is not surprising, considering the fact that the ossicular findings in patients with the CHARGE association appear to result primarily from a defective embryological development of the second branchial arch, the stapedius muscle being a second branchial arch structure, too. The same developmental defect is responsible for the abnormal course of the facial canal often seen in patients with the CHARGE association, and seen in 57% of the ears discussed here. The 100% absence of the oval window in the present study contrasts with prior radiologic findings, in which the absence was sometimes noted, but not in such a consistent way.[16] Our data are supported by histopathologic reports on 5 temporal bones with the CHARGE association describing the absence of the oval window.[20,21] As in the histopathologic reports, we noted the presence of the round window in all cases.[21] Soft tissue and osseous obliteration of the round window niche was seen in 43% of cases. Interpretation of the soft tissue obliteration of the round window should be done with caution, since 13 of 14 middle ears in our population showed mucosal disease, which was probably responsible for the soft tissue obliteration in some ears. Osseous obliteration of the round window niche is, however, never seen in normal patients and can for that reason be considered pathologic in patients with the CHARGE association. Definite proof of these observations by means of surgical correlation still needs to be produced.

Congenital facial weakness is frequent in a variety of craniofacial syndromes. It is a well-known associated feature of CHARGE, although speculation exists as to whether this feature represents a congenital facial muscle aplasia or a brain stem lesion.[22] Byerly and Pauli[23] described the likelihood of cranial (acoustic) nerve dysfunction when there are dysfunctions of other cranial nerves (eg, the seventh). They reviewed the literature and reported that 51 of 70 (73%) patients with seventh cranial nerve abnormalities had dysfunction of the acoustic nerve. Edwards et al[24] reported that in their group, facial paralysis accurately predicted ipsilateral sensorineural hearing loss. The presence of facial palsy in a patient with CHARGE association should therefore raise suspicion of possible hearing loss.

In concordance with other reports, the majority of our patients were severely to profoundly hearing-impaired.[5,23-26] The hearing loss can be conductive, sensorineural, or mixed as a result of congenital defects of the ossicular chain, eustachian tube dysfunction due to craniofacial abnormalities, or dysplasia of the bony labyrinth. Audiologic evaluation of these patients, especially at a young age, is often troublesome due to various combinations of mental retardation, young age, retarded speech and language, visual deficits, and severe hearing loss. The ABR results for air-conducted clicks often give little information because, due to the severity of the losses, responses can only be obtained at or near the intensity limits of the evoked potential test equipment. Further, click-evoked ABRs reflect merely the high-frequency range, where the greatest losses are seen in the CHARGE association. A wedge-shaped audiogram with a low-frequency conductive loss due to ossicular anomalies and a high-frequency sensorineural loss is described in the literature as being unique to the CHARGE syndrome.[25] There are no reports of progression of the hearing loss. Most patients with CHARGE association have a severe to profound hearing loss that necessitates auditory habilitation. Several factors, however, reduce the success rate. Middle ear surgery in cases of conductive hearing loss can be attempted, but is often unsuccessful because of the anomalous middle ear. In most cases, high-level amplification is necessary, but malformation and softness of the pinnae, as well as the small ear canals, often cause problems. Fluctuating hearing loss caused by middle ear effusion can create extra problems.

It is surprising that in the literature, very little is mentioned of the vestibular problems that, on the basis of radiologic observations, could be expected in these children. Anamnestically, hypotony during the first months of life and delayed motor development are mentioned, but eventually all children acquire sufficient motor abilities. The 3 main input control systems for equilibrium are the visual, the vestibular, and the proprioceptive. As the majority of these children have no developed semicircular canals, their sensory control has a dependence primarily on visual and proprioceptive input that suggests possible disturbance of locomotion in the dark. In the light, the locomotion will be optically and proprioceptively controlled. However, as the optic system has a threefold latency compared to vestibular open loop feedback control systems, retinal slip can be expected during quick movements containing high-frequency components, causing symptoms of oscillopsia and ataxia, and problems can be expected during balance sports (biking, skating, etc). These assumptions, however, are based on the experience we have with bilateral destruction of the vestibular system after childhood. It is possible that in congenital vestibular agenesis, other compensatory systems are developed.

In conclusion, the ear malformations seem to be one of the most consistent findings in patients with CHARGE association. The very characteristic pinna, the combined findings of abnormalities of the incus and stapes, absence of the oval window, absence of the stapedius muscle (with absent pyramidal eminence and tympanic sinus in most cases), and abnormalities of different parts of the inner ear (dysplastic cochlea, hypoplastic or dysplastic vestibule, and absent semicircular canals), and, as a result, the important hearing loss are characteristic and should be suspected in every child with CHARGE association. Sensory deprivation can be a significant negative factor in the development of some of the children. A severe delay in language development can represent a primary language disorder, but it can also be secondary to a hearing loss. The developmental outcome in many of these children will be influenced greatly by early management of these sensory deficits, implementation of early intervention strategies, and family support.

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EVALUATION OF THE MICROBIOLOGY OF CHRONIC MAXILLARY SINUSITIS

MERRILL A. BIEL, MD, PHD
CARL A. BROWN, MD
RICHARD M. LEVINSON, MD
GARY E. GARVIS, MD
HYMAN M. PAISNER, MD
MELVIN E. SIGEL, MD
T. MICHAEL TEDFORD, MD
MINNEAPOLIS, MINNESOTA

Chronic paranasal sinusitis is a disease that afflicts a significant percentage of the population and causes considerable long-term morbidity. With the common use of multiple courses of broad-spectrum oral antibiotics to treat this condition, there is a possibility of an alteration in the pathogens that promote a persistence of chronic sinusitis. One hundred seventy-four consecutive patients with a diagnosis of chronic maxillary sinusitis requiring an endoscopic surgical procedure were prospectively evaluated. At the time of surgery, cultures of mucopus in the maxillary sinus were aseptically obtained endoscopically and cultured for aerobes, anaerobes, and fungus. Two hundred seventeen isolates from 174 patients were obtained. Coagulase-negative staphylococci were the most common isolates (36%), followed by *Staphylococcus aureus* (25%), *Streptococcus viridans* (8.3%), *Corynebacterium* (4.6%), and anaerobes (6.4%). Although coagulase-negative *Staphylococcus* is not considered a pathogen, of the 24 isolates that had sensitivity testing performed, 13 demonstrated resistance to multiple antibiotics, including cephalothin, erythromycin, oxacillin, sulfonamides, and clindamycin. This study demonstrates that aerobic rather than anaerobic bacteria are the more common pathogens in chronic sinusitis. In addition, coagulase-negative *Staphylococcus* may be a pathogen in the disease process, and sensitivities should be obtained of this isolate for evaluation and possible treatment.

KEY WORDS — antibiotic resistance, chronic maxillary sinusitis, microbiology.

Chronic sinusitis is defined as a sinus infection that has persisted for longer than 3 months. Symptoms usually consist of nasal obstruction, facial fullness, and nasal discharge. The cause of chronic sinusitis is multifactorial. In general, chronic sinusitis is acute sinusitis that has been unsuccessfully treated, resulting in irreversible changes and edema of the sinus mucosa. This causes secondary obstruction of the sinus ostia, preventing aeration and drainage, thereby promoting further infection. Sinonasal allergies may also contribute to the development of chronic sinusitis by the same mechanisms of sinus mucosal edema and secondary sinus ostium obstruction. In addition to medical management, chronic suppurative sinusitis must be treated surgically with removal of the diseased mucosa obstructing the sinus ostia, allowing for improved sinus ventilation and drainage.

The medical management of chronic suppurative sinusitis has been based on microbiologic studies of maxillary and ethmoid sinus aspirates and mucosal biopsies. Brook[1] demonstrated anaerobes as a significant pathogen in chronic sinusitis. However, this has not been confirmed by other studies, which demonstrate *Staphylococcus aureus* and *Streptococcus* as the most common pathogens. This lack of anaerobes cultured may in part be due to the more common use of broad-spectrum oral antibiotics. As a result of the frequent use of multiple courses of broad-spectrum oral antibiotics to treat chronic suppurative sinusitis, there is a probability of an alteration in the usual pathogens that promote this disease process. The present study evaluates the microbiology of community-acquired chronic suppurative maxillary sinusitis.

METHODS AND MATERIAL

One hundred seventy-four consecutive adult patients with a diagnosis of chronic maxillary sinusitis requiring an endoscopic surgical procedure from October 1, 1991, to September 30, 1992, were prospectively evaluated. Each patient presented with a greater than 3-month history of chronic and recurring facial pain and fullness, nasal congestion, and purulent rhinorrhea. All patients had received at least 4 courses of oral antibiotics preoperatively, with the last course running for a minimum of 3 weeks immediately preoperatively. Coronal computed tomography scans were obtained preoperatively, and in all cases demonstrated obstructive chronic sinus changes with ostiomeatal unit obstruction.

At the time of surgery, cultures of the maxillary sinus were aseptically obtained endoscopically with a swab and cultured for aerobes, anaerobes, and fun-

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CORRESPONDENCE — Merrill A. Biel, MD, PhD, Ear, Nose and Throat Specialty Care of Minnesota, 2211 Park Ave S, Minneapolis, MN 55404.

TABLE 1. YIELD OF 217 MICROBIOLOGIC ISOLATES FROM 174 PATIENTS WITH CHRONIC SINUSITIS

Isolate	*No.*	*%*
Staphylococcus, coagulase-negative	78	36
26 β-lactamase–positive		
6 β-lactamase–negative		
Staphylococcus aureus	54	25
Streptococcus viridans	18	8.3
Oral flora	13	6
Propionibacterium	10	4.6
Corynebacterium	10	4.6
α-*Streptococcus*	7	3.2
Enterobacter	6	2.8
β-*Streptococcus*	3	1.4
Anaerobes	3	1.4
Aspergillus	2	0.9
Proteus	2	0.9
Pseudomonas	2	0.9
Bacteroides melaninogenicus	1	0.5
Haemophilus influenzae	1	0.5
Klebsiella pneumoniae	1	0.5
Citrobacter	1	0.5
Morganella morganii	1	0.5
Escherichia coli	1	0.5
Torulopsis glabrata	1	0.5
Streptococcus pneumoniae	1	0.5
Neisseria	1	0.5
Total	217	100

TABLE 2. ANTIBIOTIC RESISTANCE OF COAGULASE-NEGATIVE *STAPHYLOCOCCUS* SPECIES

	Total No. of Cultures	*Resistance*	
Antibiotic	*(n = 24)*	*No.*	*%*
Penicillin	24	24	100
Cephalothin	24	8	33
Erythromycin	24	6	25
Oxacillin	24	8	33
Sulfonamides	24	6	25
Clindamycin	24	2	8.3

gus by standard techniques.

All patients underwent endoscopic sinus surgery by standard techniques described by Kennedy,[2] including uncinectomy, maxillary sinusotomy, and anterior ethmoidectomy. Frank pus in the maxillary sinus was encountered in all cases, and a culture of the frank pus from the maxillary sinus was obtained in the following fashion.

Under direct endoscopic visualization of the surgically enlarged maxillary sinus ostium, a sterile rayon-tipped wire swab was introduced transnasally and placed into the maxillary sinus and the mucopus was swabbed. Great care was taken so as not to touch the tip of the swab to the nasal ala, septum, or lateral nasal wall. The culture swab used was the Baxter Culturette System consisting of a sterile rayon-tipped swab that was immediately placed in 0.5 mL of modified Stuart's bacterial transport medium. Separate culture specimens were obtained in this fashion for aerobes and fungus and were immediately transported to the microbiology laboratory for routine agar plating. Specimens for aerobic culture were plated onto chocolate, blood, colistin–nalidixic acid, and MacConkey agar. A culture in thioglycolate broth was also performed. Specimens for fungal culture were plated onto potato flake, brain-heart infusion, and inhibitory mold agars. Standard typing and identification was performed. Antimicrobial sensitivities were tested on all organisms isolated by the Kirby-Bauer method.[3] Anaerobic culture of the maxillary mucopus was performed in the same fashion, with the culture swab being placed aseptically immediately into a Cary-Blair anaerobic transport medium (Carr-Scarborough Microbiologicals, Stone Mountain, Ga) and transported to the microbiology laboratory. Anaerobic cultures were then set up on an anaerobic blood agar CDC (Centers for Disease Control) formulation and an anaerobic LKV (laked sheep blood, kanamycin, vancomycin) blood agar CDC formulation. Standard typing and identification were performed on all organisms, and sensitivities were tested again by the Kirby-Bauer method. Growth of organisms was graded semiquantitatively from 1+ to 4+.

RESULTS

Two hundred seventeen isolates from 174 patients were obtained (Table 1). Bacterial isolates were present in 94.9% of patients. Multiple organisms were cultured from 19.5% of patients. Coagulase-negative staphylococci were the most common isolates (36%), followed by *S aureus* (25%), *Streptococcus viridans* (8.3%), *Corynebacterium* (4.6%), and anaerobes (1.4%). Due to the assumption that coagulase-negative staphylococci are contaminants, most of these cultures were not typed or submitted to sensitivity testing. Of the 32 patients with coagulase-negative *Staphylococcus* that underwent typing of the bacteria, 26 were ß-lactamase–positive and 6 were ß-lactamase–negative. Twenty-four isolates of pure cultures of coagulase-negative *Staphylococcus* underwent antibiotic sensitivity testing. Of these 24, all of the isolates demonstrated resistance to penicillin, and 13 of 24 demonstrated multiple antibiotic resistance (Table 2).

DISCUSSION

Anaerobic bacteria have long been implicated as the causative pathogen of chronic sinusitis. This was established by Frederick and Braude[4] and by Brook.[11] However, studies by Karma et al,[5] Almadori et al,[6]

TABLE 3. REVIEW OF BACTERIOLOGIC EXAMINATION OF CHRONIC SINUSITIS

Authors	*Year*	*Predominant Bacteria*	*Subjects*	*Materials*	*Sinuses*
Karma et al[5]	1979	*Streptococcus viridans* *Haemophilus influenzae* Anaerobic bacteria (18%)	Adults	Biopsy, secretion	Maxillary
Brook[11]	1981	Anaerobic bacteria (100%) α-*Streptococcus* *Staphylococcus aureus* *Haemophilus influenzae*	Children	Aspirates	Maxillary, ethmoid
Almadori et al[6]	1986	*Staphylococcus*, coagulase-negative (22%) *Staphylococcus aureus* *Streptococcus pneumoniae* Diphtheroid bacillus Anaerobic bacteria	Adults	Biopsy, aspirates	Maxillary
Brook[1]	1989	Anaerobic bacteria (88%) *Staphylococcus aureus* α-*Streptococcus*	Adults	Aspirates	Maxillary
Doyle and Woodham[7]	1991	*Staphylococcus*, coagulase-negative (71%) *Staphylococcus aureus* Enterobacteriaceae *Proteus* Anaerobic bacteria (0%)	Adults	Biopsy	Ethmoid
Muntz and Lusk[8]	1991	*Staphylococcus*, coagulase-negative (44%) α-*Streptococcus* *Staphylococcus aureus* Anaerobic bacteria (6%) Fungal infections (7%)	Children	Biopsy	Ethmoid
Orobello et al[9]	1991	*Staphylococcus*, coagulase-negative (24%) *Staphylococcus aureus* *Streptococcus viridans*	Children	Biopsy, aspirates	Maxillary, ethmoid
Hoyt[10]	1992	*Staphylococcus*, coagulase-negative (47%) *Staphylococcus aureus* *Streptococcus pneumoniae* *Enterobacter*	Adults	Biopsy, aspirates	Maxillary
Present study	1993	*Staphylococcus*, coagulase-negative (36%) *Staphylococcus aureus* *Streptococcus viridans* Anaerobic bacteria (6.5%)	Adults	Biopsy, aspirates	Maxillary

Doyle and Woodham,[7] Muntz and Lusk,[8] Orobello et al,[9] and Hoyt[10] have demonstrated that anaerobic organisms were cultured in 6% or less of their series. These studies all implicated aerobic bacteria, predominantly *S aureus* and streptococcal species. These findings were consistent with the results of our study, which demonstrated a preponderance of *S aureus* (25%; Table 3[1,5-11]).

Of interest, the predominant organism cultured in all the above studies was coagulase-negative *Staphylococcus*. Despite the significant predominance of these organisms (22% to 75% of all isolates), even if they were obtained from tissue culture, this organism has always been assumed to be a contaminant, and its presence in culture has been discounted. However, coagulase-negative *Staphylococcus* has been implicated as a virulent pathogen in neutropenic and neonatal sepsis, endocarditis, and urinary tract and burn-related infections.[12-19]

As in the above studies, our study also demonstrates a predominance of coagulase-negative *Staphylococcus* (36%) in the cultures of maxillary sinus mucopus in chronic sinusitis patients. Further, 53% (41/78) of these cases had coagulase-negative *Staphylococcus* as the *only* bacterial isolate. Of the 24 patients who underwent sensitivity testing of their coagulase-negative *Staphylococcus*, 13 of these patients had organisms that were resistant to multiple antibiotics, including penicillin, cephalothin, erythromycin, oxacillin, sulfonamides, and clindamycin. These findings strongly suggest that coagulase-negative *Staphylococcus* cultured in patients with chronic sinusitis may in fact be a pathogen. These results are consistent with the results of Hoyt,[10] who found that 46% of the bacterial isolates from patients with chronic sinusitis were coagulase-negative *Staphylococcus*, of which 35% were resistant to commonly used oral antibiotics, including first- and second-generation cephalosporins.

This present study indicates that aerobic rather than anaerobic bacteria are the more common pathogens found in chronic suppurative sinusitis. Further, coagulase-negative *Staphylococcus* should be considered a potential pathogen in this disease process. We advise that all bacterial isolates of cultures of patients with chronic sinusitis should undergo the appropriate typing and antibiotic sensitivities so that the appropriate antibiotic treatment may be employed.

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MANAGEMENT OF CHRONIC SINUSITIS IN THE ADULT CYSTIC FIBROSIS PATIENT

DAVID J. HALVORSON, MD
BIRMINGHAM, ALABAMA

JOHN R. DUPREE, MD
AUGUSTA, GEORGIA

EDWARD S. PORUBSKY, MD
AUGUSTA, GEORGIA

Cystic fibrosis (CF) is an autosomal recessive disorder affecting exocrine gland function. Although CF was formerly a deadly disease of infants and children, recent improvements in antibiotics, nutritional therapy, and supportive care have extended the median survival to adulthood. Patients with CF often present with sinusitis and nasal polyposis in addition to recurrent pulmonary infections. Although the effectiveness of endoscopic sinus surgery in children with CF has been documented, the treatment guidelines and efficacy in the adult CF patient are unknown. We present a series of 16 adult patients with CF and chronic sinusitis. The majority of patients presented with nasal polyposis and concomitant pulmonary complications. Endoscopic findings are reviewed, with an emphasis on improving pulmonary function following endoscopic sinus surgery. Preliminary findings suggest that endoscopic sinus surgery improves symptoms of sinusitis and exercise tolerance and may delay the progressive respiratory failure that often affects the adult CF patient.

KEY WORDS — chronic sinusitis, cystic fibrosis, endoscopic sinus surgery, management, nasal polyposis.

INTRODUCTION

Endoscopic sinus surgery in the treatment of chronic sinusitis has expanded the role of surgical intervention, preoperative diagnosis, and postoperative care in patients with cystic fibrosis (CF). Caused by defects in a single gene on the long arm of chromosome 7 that encodes a regulatory protein for a cyclic adenosine monophosphate–regulated chloride channel, CF results in the accumulation of thick, viscous mucus associated with progressive obstruction, scarring, and destruction of excretory ducts.[1] Advances in diagnosis and treatment of patients with CF have improved the median life expectancy to approximately 30 years.[2] The extended survival is due in part to more aggressive treatment of pulmonary disease and malnutrition.[3] In effect, a tragic fatal disease of infants and children has been converted into a chronic disorder imposing significant physical, social, and economic burdens.[4] Unfortunately, as the disease progresses, 90% of patients ultimately die of respiratory failure.[5]

Recurrent pulmonary infections, the hallmark of CF, usually develop secondary to poor pulmonary toilet, progressive parenchymal disease, or concurrent upper respiratory tract infections. Chronic sinusitis has been demonstrated to be intimately associated with bacterial endobronchial infections.[6] In addition, sinusitis has been shown to affect pulmonary reactivity and often contributes to the chronicity of disease within the sinobronchial tract.[7] In patients with CF, the cycle of disease is only intensified secondary to poor mucus clearance and obstruction with a progression to pulmonary infections.

Endoscopic sinus surgery is currently advocated to alleviate nasal symptoms and improve the quality of life while hopefully reducing the pulmonary complications associated with chronic sinusitis. Multiple studies in children have recommended sinus surgery to improve nasal congestion, drainage, and cough, but eliminating the disease has not been successful.[8-10] The indications for and results of endoscopic sinus surgery in adult patients with CF have primarily been reported in those individuals evaluated for lung transplantation, who generally present with advanced CF.[11] One of the important questions to address is, at what point is surgical intervention indicated in adult patients, and does early intervention improve objective findings and subjective symptoms?

This study reviews the presentation of sinusitis and nasal polyposis in the adult CF patient and the effect of surgical intervention. In addition, it ana-

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From the Department of Surgery, Division of Otolaryngology, University of Alabama at Birmingham, Birmingham, Alabama (Halvorson), and the Department of Medicine, Section of Pulmonary and Critical Care Medicine (Dupree), and the Department of Surgery, Division of Otolaryngology (Porubsky), Medical College of Georgia, Augusta, Georgia.

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CORRESPONDENCE — David J. Halvorson, MD, Dept of Surgery, Division of Otolaryngology, 1501 Fifth Ave S, University of Alabama at Birmingham, Birmingham, AL 35233.

TABLE 1. PATIENT DATA

Patient	*Age (y)*	*Sex*	*Age at Diagnosis (y)*	*Previous Sinus Operative Procedures*
1	24	M	<1	4
2	28	M	<1	2
3	23	F	6	2
4	30	M	<1	0
5	21	F	<1	0
6	24	M	1	0
7	31	M	Birth	4
8	22	F	3	3
9	20	M	1	3
10	21	F	<1	2
11	24	M	<1	2
12	20	F	11	2
13	32	F	<1	0
14	25	F	23	2
15	25	F	2	3
16	23	F	<1	2

lyzes the outcome of surgery, addressing its effect on symptoms, recurrent polyposis, chronic bacterial colonization, and pulmonary function.

MATERIALS AND METHODS

A cross-sectional retrospective review of all adult CF patients at the Medical College of Georgia was performed from January 1993 to October 1995. "Adult" was defined as greater than 18 years of age. A limited retrospective analysis from birth to 1993 was undertaken to determine severity of illness, incidence of chronic sinusitis, and previous sinus surgery based on available medical records and patient recall.

Patients were divided into a surgical treatment group and a medical treatment group. During the 2-year study period, changes in sinus symptoms, microbiologic status, and lung function were reviewed. In addition, in all patients, microbiology of the lungs was determined by reviewing 2 separate sputum cultures obtained over a 6-month period. All patients in the study had physiotherapy on a regular basis, and bronchodilators were advocated in all patients.

In the surgical group, patients categorized symptoms of postnasal drainage, congestion, and cough as mild, moderate, or severe. Exercise tolerance was quantified as good, fair, or poor. Each patient was questioned at the time of surgery and at 3 months postoperative regarding symptoms and exercise tolerance. Microbiology of the sinuses was obtained from maxillary and ethmoid cultures taken at the time of surgery. In addition, cultures of the maxillary sinus were taken at 8 weeks postoperative. Each patient had a similar regimen of pulmonary toilet, antibiotics, and supportive care under supervision of a pulmonologist.

Pulmonary function was documented in all patients. Comparison in lung function improvement between changes in forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1) were quantitated at the time of surgery and 3 months postoperatively. Statistical difference was determined with the paired Student's *t*-test (SPSS for MS Windows 6.1).

RESULTS

The study group consisted of 16 patients over the age of 18 with documented CF. The mean age of the group was 24 ± 4 years (range 20 to 32). There were 7 men and 9 women. Patient data are shown in Table 1. The majority of patients were found to have CF within the first year of life, although 1 patient received the diagnosis at the age of 23 following recurrent pulmonary infections and nasal polyposis. Twelve of the 16 (75%) patients had at least 2 prior sinus procedures (mean 2.6 ± 2; range 2 to 4). Of the entire study group, 15 (94%) of the patients presented to either the otolaryngology service or the pulmonary service complaining of nasal obstruction or congestion and were found to have sinusitis. In addi-

TABLE 2. PULMONARY FUNCTION TESTING OF NONSURGICAL GROUP AND SURGICAL GROUP

Nonsurgical					*Surgical*				
Patient	*FVC*	*FVC % of Normal*	*FEV1*	*FEV1 % of Normal*	*Patient*	*FVC*	*FVC % of Normal*	*FEV1*	*FEV1 % of Normal*
1	3.47	68	1.78	61	2	2.60	50	0.95	23
3	4.12	80	2.14	78	5	4.42	85	2.86	69
4	3.71	72	2.05	76	6	3.58	69	1.98	48
7	4.01	87	2.12	84	8	3.01	67	2.09	57
11	4.28	96	2.34	93	9	2.38	41	0.96	21
12	3.92	92	1.87	82	10	2.76	72	1.61	52
13	3.83	76	1.61	68	14	3.27	71	1.95	61
15	4.57	89	2.35	91	16	2.37	54	1.22	48

FVC — forced vital capacity, FEV1 — forced expiratory volume in 1 second.

TABLE 3. PRESENTING SYMPTOMS

Patient	*Presenting Symptoms*	*Computed Tomography Findings*	*Endoscopic Findings*
2	Nasal congestion	Pansinusitis, nasal polyposis	Bilateral marked nasal polyposis
5	Nasal congestion, chronic purulence	Pansinusitis, nasal polyposis	Bilateral marked nasal polyposis
6	Nasal congestion, chronic purulence	Pansinusitis, nasal polyposis, frontal sinus mucosal thickening	Bilateral marked nasal polyposis
8	Nasal congestion, chronic purulence	Extensive mucosal thickening	Mucosal edema, nasal polyposis
9	Nasal congestion, chronic purulence	Pansinusitis, nasal polyposis	Bilateral marked nasal polyposis
10	Chronic purulence	Chronic mucosal thickening	Nasal polyposis obstructing osteomeatal complex
14	Chronic purulence	Nasal polyposis	Nasal polyposis
16	Nasal congestion, chronic purulence	Pansinusitis	Bilateral marked nasal polyposis

tion, 10 of the 16 (63%) of the patients were admitted for pneumonia over the 2-year study period.

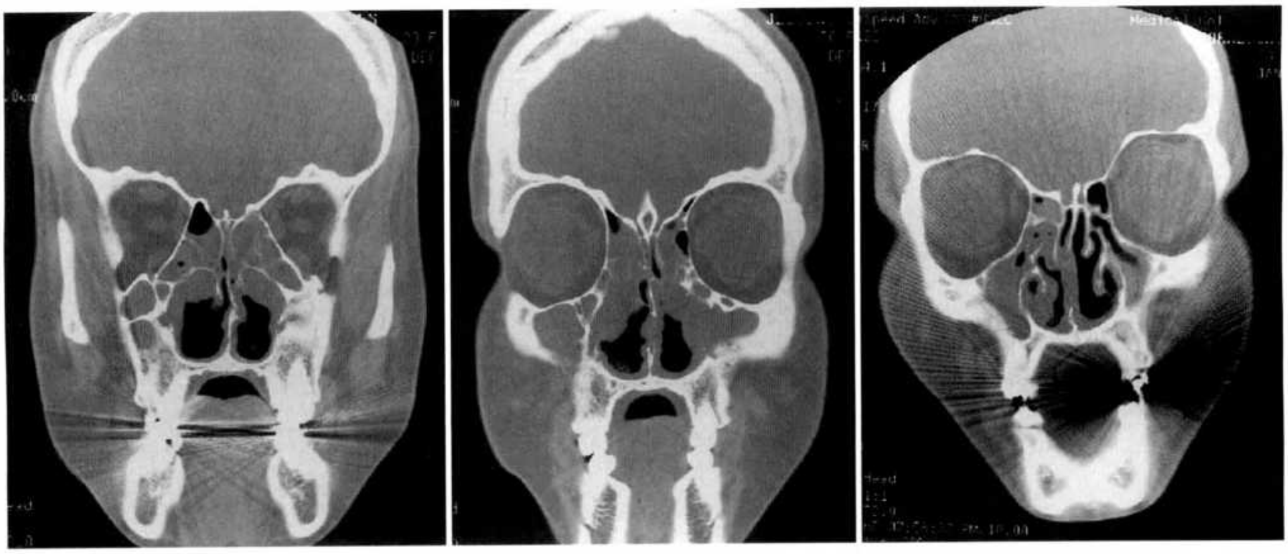
During the 2-year study period, 8 of the 16 (50%) patients had a definitive endoscopic sinus procedure. Pulmonary function testing was performed in all patients, and results for the nonsurgical and surgical groups were compared (Table 2). All of the patients in the surgical group presented with sinonasal polyposis (Table 3), and computed tomography scan findings were consistent with chronic sinusitis and nasal polyposis (see Figure). Of the patients who underwent surgery, the mean number of previous procedures was 2.7 ± 1 (range 0 to 4).

The microbiology of the entire study group is summarized in Table 4. Chronic pulmonary colonization with *Pseudomonas aeruginosa* was evident in 14 of the 16 (88%) patients. Sinus cultures grown before and after surgery are summarized in Table 5. Cultures were positive for *P aeruginosa* in 7 of 8 (88%) patients. Sinus cultures were positive for *Staphylococcus aureus* in 4 of 8 patients (50%). Postoperative culture data were available in 6 patients, and in only 2 patients was eradication of *P aeruginosa* evident. Although quantitatively reduced, *P aeruginosa* was still present as a chronic infection in 4 patients.

Each patient had a total ethmoidectomy and wide middle meatal antrostomy. During surgery, no operative complications were encountered, and all postoperative courses were uneventful. The pulmonary function following surgery was improved in all patients: FVC ($p < .01$) and FEV_1 ($p < .01$; Table 6). Subjective improvement in congestion, drainage, and cough was related by each patient 3 months following surgery, and exercise tolerance 3 months after surgery was related as good by all patients (Table 7). Every patient in the study who underwent endoscopic sinus surgery had recurrence of polyps within an 18-month period (mean months to recurrence, 14 ± 6).

DISCUSSION

As the median survival age of patients with CF increases, the indications and goals of therapy in



Three examples of computed tomography demonstrating evidence of pansinusitis and nasal polyposis.

TABLE 4. MICROBIOLOGY OF SPUTUM CULTURES

Patient	*Sputum Cultures*
1	4+ *Pseudomonas aeruginosa*
2	4+ *Pseudomonas aeruginosa*
3	3+ *Pseudomonas aeruginosa*
4	4+ *Pseudomonas aeruginosa*
5	4+ *Pseudomonas aeruginosa*
6	3+ *Pseudomonas aeruginosa*
7	4+ *Pseudomonas aeruginosa*
8	4+ *Pseudomonas aeruginosa*
9	4+ *Pseudomonas aeruginosa*
10	4+ *Xanthomonas maltophilia*
11	3+ *Alcaligenes xylosoxidans*
12	3+ *Pseudomonas aeruginosa* 1+ Burkholdericepacia
13	2+ *Pseudomonas aeruginosa*
14	4+ *Pseudomonas aeruginosa*
15	3+ *Pseudomonas aeruginosa*
16	4+ *Pseudomonas aeruginosa*

TABLE 5. MICROBIOLOGY OF SINUS CULTURES

	Sinus Cultures	
Patient	*Preoperative*	*Postoperative*
2	4+ *Pseudomonas aeruginosa*	1+ *Pseudomonas aeruginosa*
5	4+ *Pseudomonas aeruginosa*	Not available
6	4+ *Pseudomonas aeruginosa*	2+ *Pseudomonas aeruginosa*
8	4+ *Pseudomonas aeruginosa* 3+ *Staphylococcus aureus*	No growth
9	1+ *Staphylococcus aureus*	No growth
10	3+ *Pseudomonas aeruginosa* 2+ *Staphylococcus aureus*	1+ *Pseudomonas aeruginosa*
14	3+ *Pseudomonas aeruginosa*	Not available
16	4+ *Pseudomonas aeruginosa* 1+ *Staphylococcus aureus*	1+ *Pseudomonas aeruginosa*

adult patients with chronic sinusitis must be addressed. Unfortunately, the only study[11] addressing adult patients with CF analyzed the outcome of surgery in advanced disease presenting for lung transplantation. The indications for intervention, management of chronic sinusitis, prevention of bacterial colonization, and maximizing therapy prior to the development of advanced CF have not been clearly defined. One of the difficult aspects of a patient with CF approaching adulthood is that, in general, multiple sinus procedures have been performed, obscuring normal anatomic landmarks.[5] Patients with CF also have altered anatomy secondary to polyps and chronic disease. In addition, chronic sinusitis leads to poor pneumatization of the sinuses and results in hypoplasia of maxillary and ethmoid sinuses in addition to poorly developed frontal sinuses.[12] The combination of chronic disease, nasal polyposis, and altered anatomy makes surgery even more difficult in adult CF patients, and any intervention should be carefully considered.

The current study identifies a number of important observations on sinus disease in adult patients with CF. Three fourths of the entire study group underwent more than 1 sinus procedure before reaching adulthood. Those patients who did not have a history of sinus procedures presented with better pulmonary function testing (Table 2). Whether the lack of chronic sinusitis in this group was directly responsible for decreased lung disease is difficult to extrapolate, although the presence of sinusitis may have a deleterious effect. Significantly, all but 1 of the patients were found to have acute or chronic sinusitis during the 2-year study period. This supports the widely held beliefs that chronic sinusitis is not curable with surgery and that adult CF patients often present with sinusitis. The development of recurrent sinusitis may adversely affect CF patients, and intervening in the cycle of chronic sinus disease and chronic bronchopulmonary infection is critical to decreasing the risk of life-threatening pneumonia and improving the quality of life.

The surgical group consisted of 8 patients, all of whom underwent a revision sinus procedure. Four additional patients were referred for surgical intervention. Two patients refused surgery, and in the other 2 patients, the general anesthetic risk was deemed excessive. Both of these patients refused surgery with local anesthetic. In every patient who underwent a revision endoscopic sinus procedure, elimination of disease to a complete remission state was not achieved. However, after surgery, subjective improvement in symptoms of cough, congestion, and nasal obstruction was seen in all patients, and no patient required readmission for pulmonary complications. Pulmonary function and exercise tolerance were also improved after surgery, contrary to previously published data.[13] The improvement in pulmonary function testing, an objective measurement of respiratory status, may indicate that early intervention is valid when pulmonary function testing shows a decrease in FVC or FEV1. It is recognized, however, that pulmonary function testing may have significant test-retest variation. Serial postoperative tests performed in a prospective manner may provide more objective data. The improvement in exercise tolerance, al-

TABLE 6. PULMONARY FUNCTION CHANGES FOLLOWING ENDOSCOPIC SINUS SURGERY

	Preoperative				*Postoperative*			
Patient	*FVC*	*FVC % of Normal*	*FEV1*	*FEV1 % of Normal*	*FVC*	*FVC % of Normal*	*FEV1*	*FEV1 % of Normal*
2	2.60	50	0.95	23	3.22	62	1.16	28
5	4.42	85	2.86	69	4.58	88	3.11	75
6	3.58	69	1.98	48	4.06	78	2.57	62
8	3.01	67	2.09	57	3.94	88	2.35	64
9	2.38	41	0.96	21	3.21	55	1.43	31
10	2.76	72	1.61	52	3.66	97	1.93	63
14	3.27	71	1.95	61	4.23	92	2.49	78
16	2.37	54	1.22	48	3.42	78	1.86	73

though subjective, is important, as exercise testing is recognized as a correlate for functional status and has been associated with a significantly lower risk of dying.[13]

Although the data appear to indicate subjective improvement in symptoms and pulmonary status following surgery, a number of limitations of the study should be addressed. The retrospective nature of the study involves investigator bias, and a blinded controlled prospective study may provide more statistically relevant data. A strict control of other variables — pulmonary toilet, antibiotics, steroids, and supportive care — may also provide additional objective data. In addition, a direct comparison of a nonoperative group with maximal medical therapy may better delineate the effectiveness of endoscopic sinus surgery.

Unfortunately, long-term results with various procedures for nasal polyposis have been generally unsuccessful in children secondary to the inability to clear mucus and resultant chronic bacterial infection, preventing complete resolution of disease. In addition, the disease-free interval may be as short as 2 months, and more than half of all patients who undergo surgery require a revision procedure.[14-16] Each patient within our surgical group had recurrence of nasal polyposis following sinus surgery, although the mean disease-free interval was greater than 1 year. In addition to recurrent nasal polyposis, a subjective increase in nasal congestion, cough, and postnasal drainage was documented. It is evident that adult patients with CF have chronic sinusitis with a short period of symptom improvement of drainage and congestion but an eventual recurrence of polyps. There was no evidence of an intraoperative or preoperative factor that identified an earlier recurrence of polyps. Although the symptoms often returned following surgery, each patient related a subjective improvement in symptoms and supported the advocacy of endoscopic intervention when indicated. Because adult patients with CF are an even higher-risk population for pulmonary complications with chronic sinusitis, the indications and goals of surgical intervention should be outlined.

Based on the study observations, a treatment protocol has been established for sinusitis in adult patients with CF. An attempt to maximize all medical therapy is instituted, with empiric oral antibiotics as the initial treatment for a period of 4 to 6 weeks. Antibiotics are indicated when a subjective increase in drainage, congestion, or cough is related. Any pa-

TABLE 7. PREOPERATIVE AND POSTOPERATIVE SYMPTOMS

	*Drainage**		*Congestion**		*Cough**		*Exercise Tolerance†*	
Patient	*Preop*	*Postop*	*Preop*	*Postop*	*Preop*	*Postop*	*Preop*	*Postop*
2	Severe	Mild	Severe	Mild	Severe	Moderate	Poor	Good
5	Severe	Mild	Severe	Mild	Severe	Mild	Fair	Good
6	Severe	Moderate	Severe	Moderate	Moderate	Mild	Poor	Good
8	Moderate	Mild	Severe	Mild	Moderate	Mild	Fair	Good
9	Severe	Mild	Severe	Mild	Moderate	Mild	Fair	Good
10	Severe	Moderate	Severe	Moderate	Severe	Moderate	Fair	Good
14	Moderate	Mild	Severe	Mild	Moderate	Mild	Fair	Good
16	Severe	Moderate	Severe	Mild	Severe	Moderate	Poor	Good

*Severe was defined as lifestyle-impairing; moderate was defined as present but not impairing; mild was defined as approaching symptom-free.

†Poor was defined as limited tolerance; fair was defined as periods of exercise tolerance; good was defined as approaching maximal exercise tolerance.

tient with fever or pulmonary function compromise is admitted for sinus cultures and culture-specific intravenous antibiotics. Nasal steroids are usually a mainstay of therapy during an acute event and as long-term therapy. Antihistamines are generally discouraged, as they tend to increase viscosity of mucus and are only used for discomfort with a clear allergic cause. Decongestants are of variable benefit and are usually initiated on a case-by-case basis.

Unfortunately, a significant number of patients with CF fail medical treatment and are referred for surgical management. The indications for surgery may be any one of the following: 1) nasal obstruction secondary to nasal polyposis, 2) chronic purulent drainage greater than 3 months, with or without polyposis, and 3) marked decrease in pulmonary function.

Although a numerical percentage of reduction in pulmonary function has not been established, a combination of recurrent sinusitis and worsening pulmonary status demonstrated by pulmonary function testing and exercise tolerance has become one of the indications for surgery. All of the patients in the study had evidence of chronic sinusitis and nasal polyposis with a decrease in pulmonary function.

All patients are admitted for aggressive pulmonary toilet for at least 72 hours prior to surgery. Computed tomography is performed in axial and coronal images in 2- to 3-mm intervals. Realizing the increased potential for complications in revision endoscopic sinus surgery, the primary goal of surgical therapy consists of safe removal of polypoid debris, preservation of normal anatomic landmarks, and drainage of the sinuses. Removal of the polypoid mucosa is usually performed with the microdebrider. The middle meatal antrostomy is enlarged and safe removal of polyps undertaken. The important salient point to remember is that since this is not a procedure for cure, residual polyposis should be left if proper anatomic landmarks are not evident. Postoperatively, patients are started on a regimen of nasal saline irrigation and twice-daily topical irrigation with tobramycin (80 mL/250 mL normal saline).

As mentioned, the primary goal of surgery is improvement in the nasal airway and removal of chronically infected mucosa. Attempting to break the cycle of sinus and pulmonary infections may increase the life span of adult CF patients. The standard cornerstones of chest physiotherapy and bronchodilators are perhaps most important in decreasing the rate of progression of pulmonary involvement of CF.

Not surprisingly, chronic pulmonary colonization with *P aeruginosa* was present in almost every patient. Although limitations are present within the microbiology data, greater than 80% of the patients were chronically infected with *P aeruginosa* both in the sinuses and in the lower respiratory tract prior to surgery. *Staphylococcus aureus* also was evident in 50% of the sinus cultures. The data are limited, however, by the potential for *Pseudomonas* to overgrow the culture medium, obscuring the prevalence of other, slower-growing pathogens. The culture results did reveal that complete eradication of *P aeruginosa* was not attainable in all patients despite surgery and postoperative tobramycin irrigation. The data may indicate that *P aeruginosa* colonization is not affected by surgery in either the lower or upper respiratory tract and intervention should not be based solely on the presence of *P aeruginosa.*

In summary, the adult CF patient is a surgical challenge and usually presents with altered anatomy. This study demonstrates that the otolaryngologist plays a vital role in improving symptoms, exercise intolerance, and pulmonary dysfunction while aiding in the management of adult patients with CF.

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RHINOLOGY SYMPOSIA

The 18th International Symposium on Infection and Allergy of the Nose (ISIAN), the 4th Asian Research Symposium on Rhinology (ARSR), and the 3rd ASEAN Rhinology Group Congress (ARC) will be held in Manila, Philippines, February 14-18, 1999. For information, contact Gil M. Vicente, MD, Congress President, Dept of ORL-HNS, Jose Reyes Memorial Medical Center, Rizal Avenue Sta. Cruz, Manila, Philippines 1003; telephone (632) 743-6921; fax (632) 732-1077 or (632) 522-0946; e-mail isian@psohns.com; web site http://www.psohns.com/isian.

THREE-DIMENSIONAL NAVIGATION IN OTORHINOLARYNGOLOGICAL SURGERY WITH THE VIEWING WAND

WOLFGANG FREYSINGER, PHD
ANDREAS R. GUNKEL, MD
RETO BALE, MD
MICHAEL VOGELE
CHRISTIAN KREMSER, PHD
GERALD SCHÖN, MD
WALTER F. THUMFART, MD
INNSBRUCK, AUSTRIA

We report our experiences with the ISG Viewing Wand intraoperative 3-dimensional navigation device in endonasal endoscopic procedures of the paranasal sinuses, anterior skull base, and petrous bone. In the last 12 months we have routinely used the wand in 90 patients for treatment of polyposis nasi, for biopsies and removal of tumors in the nasal cavity and at the frontal skull base, for endocrine ophthalmopathy, and in 1 case for cholesteatoma. We present our computed tomography, magnetic resonance imaging, and clinical protocols that allow a precise routine use of the Viewing Wand. In all cases, the system was extremely helpful for intraoperative localization and helped to optimize surgery.

KEY WORDS — computer-assisted surgery, intraoperative navigation, ISG Viewing Wand, three-dimensional navigation.

INTRODUCTION

As is obvious for all surgical disciplines, especially otorhinolaryngologic surgery, the surgeon depends on precise localization of instruments inside the patient so as not to damage critical anatomic structures. Since the early days of medicine, there has always been a demand for knowledge of structures "inside" the patient.[1] Any historical textbook can give much information about the outcomes of procedures performed without the appropriate anatomic knowledge or exact guidance. Anatomic atlases, mostly maps of the brain, were developed and were, unconvincingly, used for preoperative planning and intraoperative guidance. The first practical application of stereotaxis and guidance for the surgeon originated in Russia in the late 19th century.[2] In the 20th century, after X-rays were widely established as a diagnostic tool, a series of mechanical devices were developed, culminating in the developments of Spiegel and Wycis.[3] Almost simultaneously with the discovery of X-rays in 1895,[4] the mathematic foundation for the calculation of computed tomographic images was laid as early as 1917.[5] With the advent of computers powerful enough to allow a practical realization of these mechanisms, computed tomography (CT) became possible.[6] This opened the door to modern intraoperative navigation and orientation.[7] Nowadays, stereotactic frames and contemporary computer-assisted navigation are widely used in neurosurgery, and these real-time navigators are on the brink of entering almost all surgical disciplines.

With reduction of the surgical approach to a keyhole, the surgeon's field of view is shrinking proportionately, and we decided to use the ISG Viewing Wand (ISG Technologies, Mississauga, Canada)[8-10] for intraoperative navigation. We report our experience with the use of the system in 90 cases and present our intraoperative implementation and protocol.

MATERIALS AND METHODS

An intraoperative setup of the ISG Viewing Wand is shown in Fig 1. Three-dimensional (3-D) digitizing is realized by a position-sensitive mechanical arm articulated to 6 degrees of freedom. The determina-

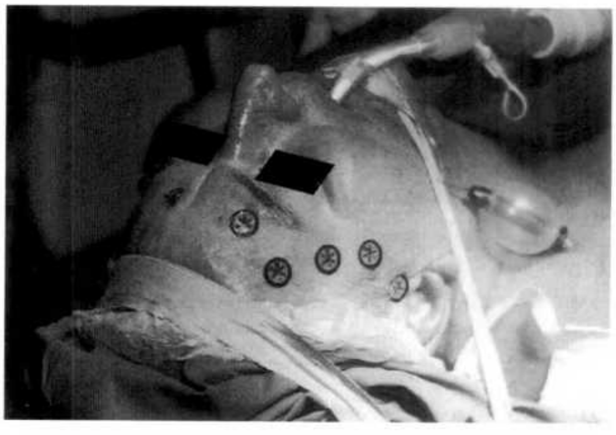

Fig 1. Intraoperative fixation of patient's head to operating table. Note positioning of radiopaque markers and skin shift induced by tape across forehead. These areas should be used for registration with caution.

From the Department of Otorhinolaryngology (Freysinger, Gunkel, Vogele, Thumfart), the Department of Radiology I, University Hospital (Bale, Schön), and the Magnetic Resonance Institute (Kremser), University of Innsbruck, Innsbruck, Austria.

CORRESPONDENCE — Wolfgang Freysinger, PhD, University ENT Department, Anichstrasse 35, A-6020 Innsbruck, Austria.

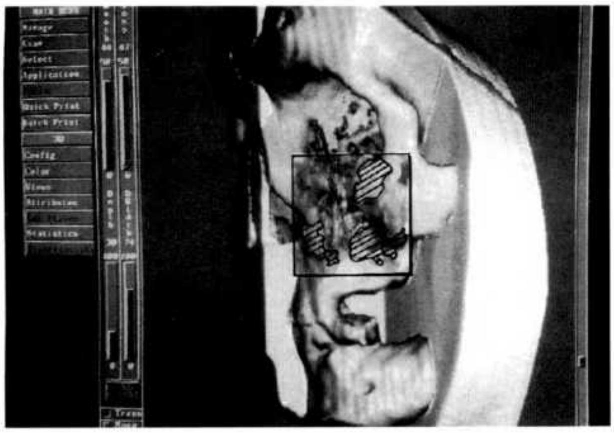

Fig 2. Typical 3-dimensional reconstruction of computed tomography scan used for surgery. Note excellent quality of image. For demonstration purposes, we have reconstructed variety of anatomic structures; polyps are shown as hatched areas.

tion of the x, y, and z coordinates and orientation of a probe mounted on the arm is done on a personal computer connected to the workstation via the serial port. We used the standard software,[8,9] version 2.0.4 beta. A variety of probes can be chosen: bent probes similar to common otolaryngology instruments, and master and slave probes. The last feature allows the system to be run in a mode in which CT and magnetic resonance imaging (MRI) studies can be used simultaneously. The 3-D reconstructions of the patient can be created on the Viewing Wand so that for routine cases the highly specialized graphic ISG Allegro workstation will not be further necessary. Features like the creation of labels, visible in all 2-dimensional and 3-D views, and various other image manipulation tools such as zooming, rotating, and moving have been shown to be very helpful for planning and intraoperative use.

Pending the completion of the clinic's network, we transfer the CT data with an optical disk to the ISG Allegro workstation, where the 3-D reconstruction is performed. With a ¼-in magnetic tape, the reconstruction is loaded into the Viewing Wand the day before surgery. Figure 2 shows a typical 3-D CT reconstruction of a patient. The MRI data are transferred from the scanner to the Allegro station online via the network.

The patients were informed about system usage and granted informed consent for the Viewing Wand to be used. We tried to schedule the 3-D CT scan for the day of hospitalization. After cleaning the skin with alcohol, we attached 6 to 8 radiopaque markers (Beekley Corp, Bristol, Conn) to the patient's skin, covering the range from the frontal sinus to the upper incisors, defining a relatively large registration volume. The markers were left in place for a maximum period of 3 days, until the day of surgery. For intraoperative registration we used only the markers that were still securely attached to the skin and had not undergone an evident change of position. In all other cases, we had a 3-D CT scan performed as a diagnostic CT scan without application of markers.

Radiologic Protocols. In most cases we used CT scans of the patient. The patient rested in supine position on the CT table and was instructed to keep the eyes closed and, to avoid motion artifacts, maintain a relaxed position. For scanning, we did not use any mechanical fixation of the patient's head and used a standard headrest. The gantry tilt was set to 0° and we performed helical scans without contrast agent, so the radiation exposure to the lenses in paranasal sinus imaging was low.[11] We used standard operating parameters of the GE High Speed Advantage scanner: 140 kV, 40 mA, standard algorithm for image calculation, large scan field of view, display field of view greater than 25 cm, table increment 3 mm, and reconstruction to 1-mm contiguous slices. Every third image was printed on film for diagnosis and backup intraoperative orientation. Data acquisition took approximately 1 minute.

The 3-D MRI images were acquired on a Siemens Magnetom Vision 1.5-T scanner in sagittal orientation with a cubelike voxel structure, yielding typically 150 images. The parameters for the 3-D sequence used were T1-weighted MPRAGE sequence, flip angle 12°, TR = 9.7 ms, TE = 4 ms, TI = 300 ms, 146 3-D partitions, sagittal slices, 256 × 256 pixels, 0.94-mm edge, 1.2-mm slice thickness, and field of view 240 × 240 mm. The whole imaging process was completed in less than 5 minutes.

Intraoperative Protocol. During system start-up the patient was prepared for surgery in the supine position, and the probes were calibrated with the mechanical arm mounted on the Viewing Wand's cabinet. Upon completion, the probes were sterilized. Once the patient was anesthetized, his or her head was firmly attached to the surgical table with 2-in surgical tape. We ran the tape across the forehead and the philtrum and immobilized the head, as shown in Fig 1. The mechanical arm was mounted onto the T-rail of the operating table, while not turning the computer system off; the patient's skin was prepared, and the operating field and the arm were sterilely draped.

The software provides 3 different registration procedures that we use alternatively: reference markers, anatomic landmarks, and a supplementary surface fitting algorithm that we use in the absence of CT markers or well-defined anatomic landmarks. Typical landmarks are left and right inner canthi, in-

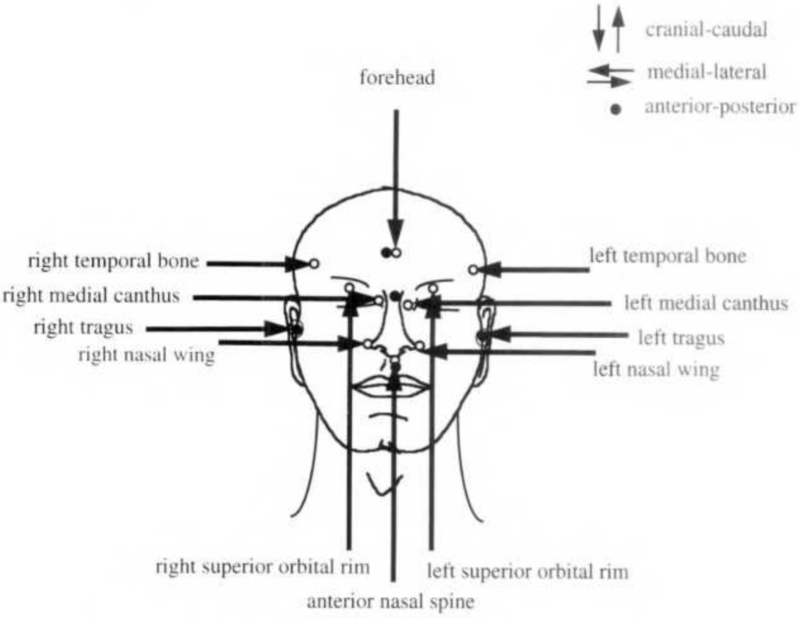

Fig 3. Schematic drawing of intraoperative clinical protocol used to determine accuracy of registration. Arrows and open circles symbolize orientation and placement, respectively, of Viewing Wand's probe on patient's head. Medial and paramedian probe orientations perpendicular to skin surface are shown as filled circles.

cisure between crus helicis and tragus, anterior nasal spine, and nasion. In brief, registration of the patient was achieved by touching well-defined features (markers, anatomic landmarks) of the patient with the probe and correlating them to their respective positions in the CT slices on the screen.

Next, we performed a quality control of the registration. The surgeon positioned the probe on selected anatomic locations (Fig 3). Each point was touched without compressing the skin, in the 3 anatomic planes (anteroposterior, frontal, sagittal). We used the trajectory 0° view, similar to an ultrasound view, and touched points in the medial plane (forehead, nasion, anterior nasal spine) and the paramedian planes (medial canthi, the most lateral aspects of the alae, and the tragi). We recorded the in-out, left-right, and up-down differences between the actual probe position and the displayed one. This yielded the surface registration accuracy of the Viewing Wand. A registration was accepted if this value was less than 3 mm. Finally, the surgeon pointed to anatomic structures in the nasal cavity and evaluated the registration inside the patient by comparing the actual position with the calculated one.

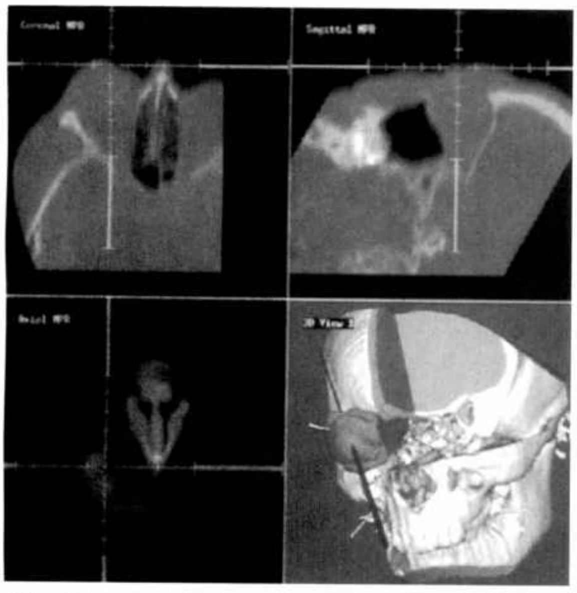

Fig 4. Intraoperative screen-shot of Viewing Wand navigation system showing axial, coronal, and sagittal CT images, and 3-D reconstruction of patient undergoing videoendoscopic orbital decompression. Exophthalmos of right eye is clearly demonstrated by graphic capabilities of system.

RESULTS

With our clinical protocol we are now routinely obtaining registration errors of approximately 2 mm. Evaluating all performed procedures by averaging the system's root mean square values yields 1.1 mm. Critical evaluation of the registration points, the procedure itself, and repeating the registration procedure may be necessary to reach an improved patient-image registration. It should be noted, though, that in certain cases we encountered inaccuracies of the perioperative patient-image registration as large as 5 mm. This rather large deviation was detected by application of our intraoperative protocol (Fig 3) and could in most cases be attributed to errors made during the registration procedure, ie, inadvertent movements of the reference markers attached to the skin, bad definition of the anatomic landmarks chosen, differences between CT position and operating table positioning of the patient with very soft facial tissue, and inaccuracies in the patient-image correlation procedure itself. The navigation system does provide information about "suspicious" registration

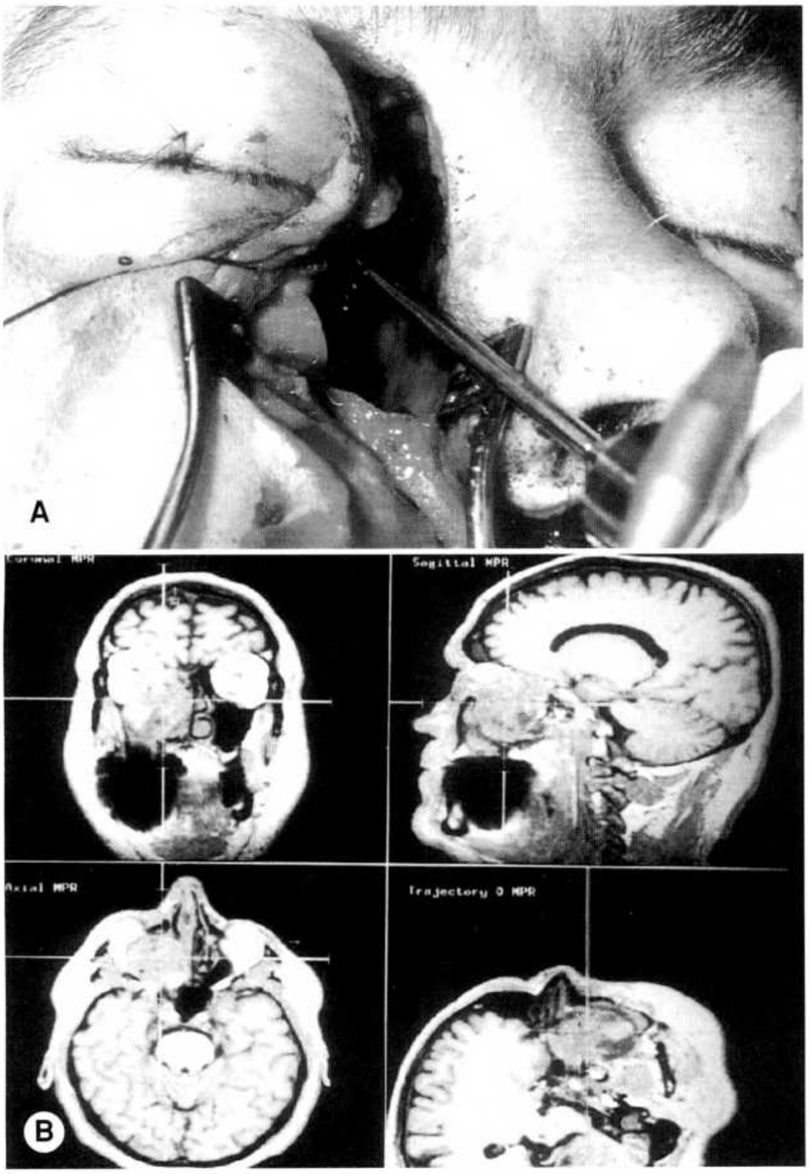

Fig 5. Patient with adenocarcinoma of paranasal sinuses involving periorbital fascia and apex of orbit. A) Placement of Viewing Wand's probe tip on apex of right orbit from exterior. B) Intraoperative screen-shot showing position of probe's tip as determined by Viewing Wand navigation system. Magnetic resonance imaging sequence used for these images provides detailed information about cranial arterial tree and allows precise intraoperative differentiation of tumorous tissue. Dental filling caused signal annihilation of right maxillary region (see text).

points; improvement of registration can be achieved by deselecting these points and recalculation or repetition of the entire registration procedure.

In the following, we briefly present 2 typical operations performed with the ISG Viewing Wand. The first was an endonasal video endoscopic decompression of a typical endocrine ophthalmopathy. After application of general anesthesia, the patient was positioned and mechanically immobilized on the operating table. Registration with fiducial markers gave a mean error of 1.5 mm, and evaluation of the registration showed an average deviation of about 2 mm. After septal correction and removal of ethmoidal cells and the lamina papyracea, the periorbital fascia was incised to decompress the orbit. The probe of the Viewing Wand was inserted in the direction of the apex of the orbit to check how far the orbit could be opened posteriorly without putting the optic nerve at risk. Figure 4 shows an intraoperative evaluation of the endocrine orbitopathy. The procedure was completed without complications.

The second case was the resection of a large adenocarcinoma, filling the right nasal cavity and eroding the medial orbit and the frontal skull base. We used our clinical protocol to evaluate the marker-based registration and started the open procedure. We established landmarks for recovering registration in case of movements of the patient's head, which did not occur. We beneficially used the system to determine how far dorsally and cranially tumorous tissue could be removed safely, without violating the optic nerve and entering the anterior cranial fossa. Figure 5A shows an intraoperative photograph of the probe inserted into the large opening created to remove the tumor. Figure 5B shows the correlating position of the probe as displayed by the

Viewing Wand.

DISCUSSION

We use axial CT scans mainly to avoid the typical artifacts of dental fillings, and frequently use the Viewing Wand's image manipulation and display capabilities for preoperative planning. With CT scanning, reliable 3-D data sets are produced routinely. For 3-D MRI images, accurate shimming (ie, magnetic field correction) is essential. In addition, it is important to avoid signal annihilation due to metallic objects (even makeup). Areas without signal can easily be recognized, but they can destroy the entire radiologic image. The intraoperative use of MRI images revealed a high symmetry of the employed magnetic field. However, due to the difficulties in registering a patient to his or her MRI images and the typical errors introduced by the registration process itself, it is hardly possible to determine the definitive contribution of the inhomogeneities of the magnetic field to the overall calibration error. So far, our experience with patients with diagnostic MRI scans is too small to give a definitive clue about the influence of the imaging parameters.

On 2 occasions the intraoperative navigation system failed. In the first case, unnoticed motion artifacts destroyed the 3-D CT data set so that registration could not be performed. In the second case, a radical mastoidectomy for cholesteatoma, we did not provide enough calibration points or a large enough calibration surface. Four times, upon improving registration with the surface fitting algorithm, the system halted. The safe architecture of the software, though, allowed resumption of the use of the Viewing Wand with the stored marker/anatomic landmark registration, and only a minimum amount of time was required for automatic system recovery. The tape fixation has proven to be acceptable for most procedures,[9] if the exerted forces are not too large. Intraoperatively, the software allows restoration of the registration, if necessary, but of course, realignment and quality checks are time-consuming. On the other hand, we deem invasive fixation methods,[8] such as stereotactic headframes or insertion of mini-screws to the skull, unacceptable for the type of procedures we report in this paper. Therefore, we have developed a rigid, noninvasive, universal head-holder that will allow a reliable fixation of the patient during radiologic imaging, avoiding motion artifacts and permitting exact repositioning on the operating table.[12] Our clinical protocol has been shown to be easy to handle, and allows an efficient and reliable check of the patient-image registration.

In all cases operated on, usage of the Viewing Wand with an intraoperative registration accuracy of less than 3 mm did not cause any intraoperative complications. On the contrary, the navigation system allowed us to extend the limits of safe surgery and helped to avoid iatrogenic complications in delicate areas. So, surgery was relatively safe in close proximity to the optic nerve — as described here — or at the posterior wall of the sphenoid sinus. Further, the use of 3-D intraoperative navigation helped to greatly reduce the operating time. This cannot be quantified, unfortunately, but, as can be inferred from the 2 exemplary cases, by providing exact positional information and delineating the outlines of the tumors to be resected, 3-D navigation significantly aided the surgeon in performing a most radical and safe surgery. The substantial investment cost and the human resources needed for using this technology are justified by the optimized therapy that can be delivered to the patient.

CONCLUSIONS

We have successfully used the ISG Viewing Wand in our department for 90 otolaryngological operations on the paranasal sinuses and the anterior skull base. The Wand proved to be a reliable and robust pointing device, providing the surgeon with exact 3-D information in proximity to vital structures. The only disadvantage of this system is the mechanical articulated arm introduced into the surgical field. This drawback, though, is by far outweighed by the benefits arising from system usage. We are currently developing extensions to the system to attach surgical instruments to the articulated arm, and further studies concerning application of the Viewing Wand to other surgical procedures are under way, eg, 3-D computer-assisted interstitial brachytherapy of head and neck tumors. To date, we have performed approximately 200 3-D–assisted procedures in otolaryngology with the mechanical arm and an infrared sensing system.

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OTOPLASTY AND RECONSTRUCTION OF AURICULAR DEFECTS

The International Course on Otoplasty and Reconstruction of Auricular Defects will be held in Lübeck, Germany, March 21-22, 1999. For information, contact PD Dr Dr R. Siegert/M. Haase, Department of Otorhinolaryngology and Head and Neck Plastic Surgery, Medical University of Lübeck, Ratzeburger Allee 160, D-23538 Lübeck, Germany; telephone +49-451-500-3189; fax +49-451-500-4192; e-mail: Sigert@medinf.mu-luebeck.de.

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RECOVERY OF ANAEROBIC BACTERIA FROM A POST-TRAUMATIC NASAL SEPTAL ABSCESS

A REPORT OF TWO CASES

ITZHAK BROOK, MD, MSC
WASHINGTON, DC

This report presents 2 children (boys, 11 and 9 years old) who had post-traumatic nasal septal abscesses. Cultures for aerobic and anaerobic bacteria revealed *Peptostreptococcus magnus* and a ß-lactamase–producing *Prevotella intermedia* in the first patient, and *Peptostreptococcus anaerobius, Streptococcus intermedius,* and *Prevotella melaninogenica* in the second patient. Anaerobic bacteria may play a role in nasal septal abscesses.

KEY WORDS — abscess, nasal septum, *Peptostreptococcus, Prevotella.*

INTRODUCTION

Nasal trauma, which is the most common of all facial injuries, may cause nasal septal hematoma that can become infected and may rarely lead to abscess formation.[1] Nasal septal abscesses can lead to nasal deformities, as well as to extension of the infection into the central nervous system.[1,2] The organisms isolated from nasal abscesses in previous reports were *Staphylococcus aureus, Haemophilus influenzae,* and *Streptococcus pneumoniae.*[2,3] The lack of recovery of any anaerobic bacteria from these abscesses may be due to utilization of inadequate methods for their recovery. This report describes the recovery of anaerobic bacteria from nasal septal abscesses in children.

CASE REPORTS

Case 1. An 11-year-old boy who sustained trauma to the nose during skating 13 days earlier presented with intensifying local nasal pain, difficulty of breathing through the nose, and a temperature of 38.1°C. Abnormal findings were only found in the nasal area: a tender septal mass obstructing most of the nasal passage, and red hyperemic nasal mucosa. The white blood cell count was 15,000/mm^3 (73% neutrophils, 15% lymphocytes, 7% monocytes, and 5% bands). Radiography showed no septal fracture or dislocation. The right side of the mass was aspirated after the nasal mucosa was disinfected with Betadine and alcohol, and 2 mL of purulent, bloody fluid was collected.

A bilateral incision and drainage was done afterward under local anesthesia, and an additional 3 mL of fluid was collected. Gram-positive cocci in chains and weakly stained gram-negative rods were observed in Gram-stained smears of the fluid. The aspirated fluid was plated within 15 minutes of collection in media supportive of the growth of aerobic and anaerobic bacteria. *Peptostreptococcus magnus* and *Prevotella intermedia* were the only organisms recovered from the fluid. ß-Lactamase production was detected in *P intermedia* by the cefinase methodology.[4] Drains and nasal packs were inserted bilaterally, and the patient received oral clindamycin hydrochloride 25 mg/kg per day for 21 days. The patient's fever subsided within 36 hours, and the pain and swelling disappeared gradually over a 6-day period. He eventually made a complete recovery.

Case 2. A 9-year-old boy who traumatized his nose 11 days earlier presented with increasing nasal pain, difficulty in breathing through the nose, and a temperature of 38.7°C. Abnormal findings were only found in the nasal area: a septal mass completely obstructing the nasal passage, and red hyperemic nasal mucosa. The white blood cell count was 17,500/mm^3 (69% neutrophils, 11% lymphocytes, 8% monocytes, and 22% bands). Radiography showed no septal fracture or dislocation. The right side of the mass was aspirated after the nasal mucosa was disinfected with Betadine and alcohol, and 3.5 mL of purulent, bloody fluid was collected.

A bilateral incision and drainage was done afterward under general anesthesia, and an additional 5 mL of purulent, foul-smelling fluid was collected. Gram-positive cocci and gram-negative rods were observed in Gram-stained smears of the fluid. The aspirated fluid was plated within 10 minutes of collection in media supportive of the growth of aerobic and anaerobic bacteria. *Peptostreptococcus anaerobius, Streptococcus intermedius,* and *Prevotella mel-*

From the Department of Pediatrics, Georgetown University School of Medicine, Washington, DC.
CORRESPONDENCE — Itzhak Brook, MD, MSc, PO Box 70412, Chevy Chase, MD 20813-0412.

aninogenica were the only organisms recovered from the fluid. Drains and nasal packs were inserted bilaterally, and the patient received oral amoxicillin–clavulanate potassium 40 mg/kg per day (for amoxicillin) for 21 days. The patient's fever subsided within 48 hours, and the pain and swelling disappeared gradually over a 5-day period. He eventually had a complete recovery.

DISCUSSION

This report describes for the first time the recovery of anaerobic bacteria from a nasal septal abscess. The recovery of *Peptostreptococcus* spp, microaerophilic streptococci, and *Prevotella* spp from this infection is not surprising, as these organisms are part of the oropharyngeal and nasal mucosa bacterial flora.[5] These organisms have also been isolated from other oropharyngeal infections in children, such as peritonsillar, retropharyngeal, and parotid abscesses and cervical lymphadenitis.[6] Prospective studies are warranted to further investigate the role of anaerobic bacteria in nasal septal abscesses in children.

A proper management of nasal septal abscess mandates prompt surgical drainage and antimicrobial therapy. It is recommended that pus or fluid collected from nasal septal abscesses be cultured for aerobic and anaerobic bacteria. This is important, because over half of the anaerobic gram-negative bacilli isolated from oropharyngeal infections can be resistant to penicillins through the production of ß-lactamase.[7] The presence of penicillin-resistant anaerobic organisms may require the use of agents effective against these bacteria. These antimicrobials include metronidazole, chloramphenicol, clindamycin, imipenem, cefoxitin, and the combination of a penicillin and ß-lactamase inhibitors.[8] Although ß-lactam–resistant penicillins (ie, methicillin) are active against *S aureus,* they are not effective against ß-lactamase–producing anaerobic bacteria.

ACKNOWLEDGMENT — The author acknowledges the secretarial support of Helen Wojciechowski.

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PARTIAL CRICOTRACHEAL RESECTION FOR SEVERE PEDIATRIC SUBGLOTTIC STENOSIS: UPDATE OF THE LAUSANNE EXPERIENCE

PHILIPPE MONNIER, MD
FLORIAN LANG, MD
MARCEL SAVARY, MD
LAUSANNE, SWITZERLAND

Until recently, severe pediatric subglottic stenosis (SGS) has been treated almost exclusively by laryngotracheoplasty procedures. Even in the most experienced centers, the results of single-stage operations for Cotton's grade III and IV stenoses have been disappointing. This paper reports our experience on 31 partial cricotracheal resections for severe SGS in infants and children. The stenosis was congenital in 6 cases and acquired after prolonged intubation in 25 cases. Twenty-seven patients were tracheotomy-dependent at the time of surgery. Twenty-two cases were classified as grade III and 9 cases as grade IV stenoses according to Cotton. The decannulation rate was 97% (30 of 31 cases) after an open procedure. There were no fatalities and no lesions to the recurrent laryngeal nerves, but there was 1 complete restenosis. Twenty-seven patients show no exertional dyspnea, 3 have a slight stridor with some dyspnea while exercising, and 1 patient is not decannulated. The voice is normal in 21 cases, a dysphonia is present in 9 cases, and the patient with complete restenosis acquired an esophageal voice. Postoperative follow-up is longer than 10 years in 8 cases and longer than 5 years in an additional 6 cases. All patients who reached adulthood show normal growth of the larynx and trachea. Considering the excellent results obtained in this consecutive series of 31 cases, partial cricoid resection with primary thyrotracheal anastomosis should be considered an important treatment option for severe SGS in infants and children.

KEY WORDS — children, cricotracheal resection, infants, laryngotracheoplasty, subglottic stenosis.

INTRODUCTION

Until recently, specialists concerned with pediatric airway problems have been reluctant to use a partial cricoid resection with primary tracheal anastomosis for the treatment of severe subglottic stenosis (SGS) in infants and children. The reasons have included the risk of a possible dehiscence at the site of the anastomosis, the likelihood of injury to the recurrent laryngeal nerves (RLNs), and interference with normal growth of the larynx.[1-3] Moreover, the necessity of changing the surgical concept of an enlargement laryngotracheoplasty to that of a resection of the stenotic segment with end-to-end anastomosis may explain the difficulty otolaryngologists have had in modifying their approach.[4-6]

Conversely, procedures for laryngotracheoplasty using a midline anterior incision with anterior, posterior, or combined costal cartilage grafts have seemed simpler and safer.[7] Despite numerous refinements of the procedure first described in 1919 by Hett,[8] it must be admitted that even in the best hands, the rate of success of single-stage laryngotracheoplasty for severe (Cotton's grade III to IV) SGS has been disappointing.[4] Even in staged operations in which the tracheotomy is maintained for several days or weeks, a second open procedure may be necessary in as many as 20% to 50% of the most severe cases.[1,2] These inconstant results led us (for the first time in 1978) to use a partial cricoid resection with primary tracheal anastomosis for a severe SGS in a 10-year-old child. By that time, we already had good success with this type of surgery in adult cases, and we were well aware of the outstanding results published in the literature by several other authors who had performed cricotracheal resections in large numbers of adults.[9-12]

Our first results in 15 pediatric cases were published in 1993.[13] The rate of success for severe pediatric stenoses (Cotton's grades III and IV) was 93% (14 of 15 cases). Since then, we have performed surgery on 16 new pediatric cases without a single failure. Long-term follow-up (>10 years) in 8 cases has shown normal development of the laryngotracheal airway.

In this paper, we present an update on our experience with 31 cases of partial cricoid resection with primary tracheal anastomosis in infants and children.

MATERIALS AND METHODS

From March 1978 to September 1996, 88 pediatric patients with laryngo-subglottic stenoses were seen in the authors' department. Thirty-seven (42%) Cotton grade I stenoses were treated endoscopically in suspension microlaryngoscopy with the carbon

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From the Department of Otolaryngology–Head and Neck Surgery, University Hospital, Lausanne, Switzerland.

CORRESPONDENCE — Philippe Monnier, MD, Dept of Otolaryngology–Head and Neck Surgery, University Hospital, CHUV, 1011 Lausanne, Switzerland.

dioxide laser and gentle bougienage if necessary. Six (7%) minor stenoses had recovered spontaneously after resolution of an acute episode of dyspnea due to an upper airway infection. Forty-five (51%) cases required an open surgical procedure. Of this latter group, 31 patients with severe (Cotton's grades III and IV) SGS underwent a partial cricoid resection with primary thyrotracheal anastomosis.

Operative Procedure. If the patient is tracheotomy-dependent, then the surgical procedure is carried out under general anesthesia via the tracheotomy. A necklace incision is made with a crescent-shaped skin resection around the tracheostoma when the latter is to be resected during the same surgical procedure.

If the patient has no tracheotomy, then the SGS is dilated by endoscopic bougienage at the time of induction of anesthesia, and the airway is catheterized with the largest possible endotracheal tube that will pass through the dilated stenotic segment. The trauma induced by the dilation has no consequence on the final result, because the subglottic area is fully resected during the surgical procedure.

The strap muscles are separated aside from the midline, and the trachea is dissected by staying in close contact with the cartilaginous rings to avoid any lesion to the RLNs. Cranially, the dissection is carried out up to the first tracheal ring. At the level of the cricoid, a vertical anterior midline incision of the perichondrium is made, and the cricoid cartilage is dissected in a subperichondrial plane laterally down to the anterior border of the cricothyroid joint.

Because the RLNs always run posteriorly to the cricothyroid joints, there is little danger of injury from this technique. The superior resection line starts at the inferior border of the thyroid cartilage. The lateral section of the anterior arch of the cricoid is made just anterior to the cricothyroid joint, thus exposing the cricoid plate completely. Usually, the latter is thickened by a submucosal fibrosis constituting the posterior aspect of the SGS. This pathologic tissue is fully resected and the cricoid plate is denuded of its mucosa almost up to the cricoarytenoid joints. If necessary, a diamond bur is used to widen the cricoid plate posteriorly and laterally, as it is done routinely in severe congenital cartilaginous stenosis of the cricoid ring. This allows the first normal tracheal ring used for the anastomosis to adapt itself to the narrower subglottic space.

Contrary to the technique proposed by Pearson et al[12,14] in adults, in which the normal tracheal ring is adapted to the smaller subglottic area by plicating the membranous trachea, we perform in children a partial inferior midline thyrotomy up to the level of the anterior commissure, without transecting it. The inferior border of the thyroid cartilage is spread apart; this maneuver is easily performed in children, whose thyroid cartilage is usually soft and pliable. In this way, the subglottic lumen is enlarged considerably, while the anterior commissure is kept intact, thus preserving a good voice.

The triangular defect is filled in with a mucosa-lined cartilaginous wedge obtained from the first normal tracheal ring below the resected stricture. The first 3 stitches are placed between the mucosa of the posterior subglottic area and the membranous portion of the trachea with 6.0 Vicryl interrupted sutures. As it is extremely difficult to tie the knots from the outside, they are tied inside the lumen. All other stitches are placed submucosally through the tracheal and cricoid cartilages laterally and through the tracheal and thyroid cartilages anteriorly. The use of jet ventilation through a small catheter is very helpful to improve local visualization during this stage of the operation.

One should be very careful not to tie an RLN with a stitch at the posterolateral edge of the cricoid plate. Before completing the anastomosis anteriorly, the cartilaginous wedge used to fill in the triangular defect of the anterior subglottis is cut to the appropriate size. It is then sutured in place by 3 stitches. A further stitch is placed at the inferior and posterolateral edge of the cricoid plate to secure the trachea in place and release the tension at the site of the anastomosis (Fig 1).

In cases in which a severe SGS is associated with a posterior glottic stenosis, blocking the vocal cords in the midline, a direct palpation of the arytenoids is done during the preoperative endoscopic workup. If both cricoarytenoid joints are blocked, then a laser arytenoidectomy is performed in a second stage procedure later in life. If the interarytenoid fibrosis has not completely fixed the arytenoids, then the scar tissue is resected up to the transverse interarytenoid muscle. The mucosal surface is restored with a pedicled flap of the membranous portion of the trachea, which is obtained by resecting 2 more tracheal rings distally. This is the best option if the tracheal segment to be resected is not too long. As it is extremely difficult to suture the mucosal flap laterally and to the lower edge of the cricoid plate, only three 6.0 Vicryl sutures are placed at the posterior commissure of the larynx to fix the flap proximally.

To avoid any bulging over the cricoid plate, fibrin glue is used to secure the mucosal flap in place. Both compounds of a homologous fibrin glue are applied

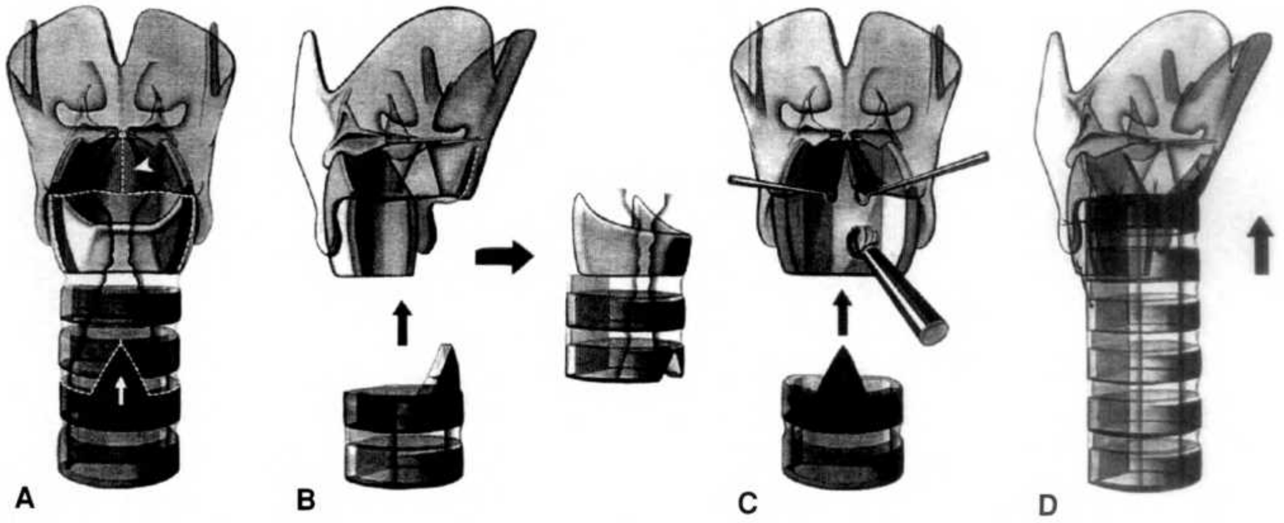

Fig 1. Diagram of partial cricoid resection with primary thyrotracheal anastomosis (see text for detailed description). A) Frontal view showing superior resection line (white dotted line) made at inferior border of thyroid cartilage and lateral section of cricoid arch made just anterior to cricothyroid joints. Posteriorly, cricoid plate is denuded of its mucosa almost up to level of cricoarytenoid joints. Partial inferior midline thyrotomy is aimed at increasing subglottic lumen, while keeping normal voice (arrowhead). Inferior resection line (white dotted line) is made at first normal tracheal ring; anteriorly, triangular mucosa-lined cartilaginous wedge is kept attached to tracheal ring to fill in anterior subglottic defect resulting from inferior midline thyrotomy (arrow). B) Oblique view showing cricotracheal segment to be resected. C) Frontal view after resection of stenotic segment. Before adapting first normal tracheal ring to narrow subglottic space, scar tissue constituting posterior aspect of stenosis is fully resected and cricoid plate is enlarged with diamond bur. Wings of thyroid cartilage are spread apart to increase subglottic lumen. Anastomosis is made posteriorly first, with three 6.0 Vicryl sutures tied inside lumen. Cricotracheal and thyrotracheal sutures are placed submucosally and triangular wedge of cartilage is secured in place between thyroid alae with Vicryl sutures. D) Oblique view showing result after completion of anastomosis. Notice that oblique suture is placed between third tracheal ring and cricoid plate to release tension on suture line.

simultaneously with a double syringe device to the raw inner surface of the cricoid plate, and the flap itself is firmly pressed onto it (Fig 2).

The treatment of the tracheostoma site depends on the location and extension of the stenosis. Two normal tracheal rings must be left intact between the anastomosis and the stoma site if one wishes to keep the initial stoma. In cases in which this is not possible (high tracheostoma, significant suprastomal collapse), the entire stoma area is resected with the stenosis. If the glottis is not involved in the stenosis (pure SGS), it is preferable to avoid a new tracheotomy distally. The child is kept intubated for 1 week and is extubated in the intensive care unit. The youngest infant treated successfully by this technique was 3 months old. In glotto-subglottic stenoses, the chances are not good of extubating the patient after 1 week without problems. In these cases, a new tracheotomy is placed distal to the site of the anastomosis.

In infants and children, the larynx is situated high in the neck. The elasticity of tissues allows the resection of the cricoid and at least 5 of the tracheal rings without the need for a supralaryngeal release or hilar mobilization. In these cases, the dissection of the trachea should only be performed anteriorly and laterally; the membranous portion should remain attached to the anterior wall of the esophagus to preserve the vascular supply.

Patients. From August 1978 to September 1996, 31 infants and children suffering from a severe (Cotton's grades III and IV) SGS underwent a partial cricoid resection with primary thyrotracheal anastomosis.

The cause of the stenosis was congenital in 6 cases. Of the remaining 25 patients, who had acquired stenosis after prolonged intubation, 6 cases probably resulted from mixed causes, namely, a mild asymptomatic congenital stenosis aggravated by an intubation.

Twenty-eight cases were referred to our center: 13 cases for primary care and 15 cases after failed laryngotracheoplasty or endoscopic procedure (laser and/or dilation). Twenty-seven patients were tracheotomy-dependent at the time of surgery; only 4 infants had surgery early enough to avoid a primary tracheotomy. Due to the length of the stenosis or to the presence of a tracheotomy, a segment of cervical trachea (1 to 6 rings at most) had to be resected with the subglottic area in 25 cases. Six patients had surgery during the first year of life and 14 before age 3

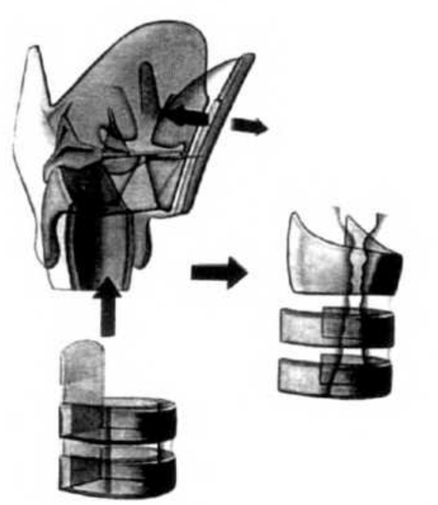

Fig 2. Diagram of cricotracheal resection combined with treatment of posterior glottic stenosis. Principle of operation is essentially identical to that of cricotracheal resection. If posterior glottic stenosis is present without bilateral cricoarytenoid ankylosis, scar tissue is fully resected up to transverse interarytenoid muscle through temporary midline thyrotomy. Pedicled flap of posterior membranous trachea is obtained by resecting 1 or 2 more tracheal rings, and upper edge of posterior mucosal flap is sutured to posterior commissure of larynx. Fibrin glue is used to fix mucosa to cricoid plate. Laryngo-subglottic stent is left in place for 6 weeks.

years. None of the remaining 16 patients was older than 13 years of age at the time of surgery.

According to Cotton's new classification,[15] all cases were either grade III (22 cases) or grade IV (9 cases) stenosis.

Preoperatively, the voice was absent in 24 tracheotomized patients, who presented either with a complete SGS (9 cases) or with a subtotal obstruction (residual pinhole opening of the airway in 15 cases). By subjective evaluation, the voice was considered normal in 4 cases, and dysphonia was present in 3 cases. It must be emphasized, however, that a precise preoperative evaluation of the voice is always difficult in a tracheotomized child with severe SGS.

Postoperative Care and Follow-up. After surgery, all infants and children stay under close supervision in the intensive care unit. Broad-spectrum antibiotics are given to all patients for 10 days starting the day of surgery, whereas corticosteroids are given only to nontracheotomized patients on the day of extubation and on the following days if necessary.

Tracheotomy-dependent children may return to a routine care unit as soon as frequent cleansing of the cannula is no longer necessary. All patients without tracheotomy are sedated and kept intubated for a minimum of 5 to 7 days. Extubation is carried out under general anesthesia after a control endoscopy. If the glotto-subglottic area is still swollen or if there is granulation tissue on the suture line, then the child is reintubated for 2 more days. A gentamicin-corticosteroid ointment is placed around the endotracheal tube to help diminish the inflammatory reaction. If the glotto-subglottic airway permits extubation, then the endotracheal tube is withdrawn under light sedation to facilitate weaning of the child from the ventilator.

TABLE 1. PREOPERATIVE AND POSTOPERATIVE DEGREE OF SUBGLOTTIC STENOSIS IN 31 PEDIATRIC CASES (NEW COTTON CLASSIFICATION)

Grade	*Preoperative*	*Postoperative*
I (<50%)	0	30 (97%)
II (51%-70%)	0	0
III (71%-99%)	22 (100%)	0
IV (No lumen)	9 (100%)	1

Classification from Myer et al.[15]

Endoscopies are routinely performed at 3 weeks and 3 months to optimize the final result by removing granulation tissue, vaporizing a partial web at the site of the anastomosis with the carbon dioxide laser, or performing a dilation by gentle bougienage with Savary-Gilliard dilators.

RESULTS

Postoperatively, a normal lumen was formed in 28 cases, a better than grade I stenosis (80% and 70% size lumens) in 2 cases, and a complete restenosis in 1 case (Table 1[15]). This last patient, a 13-year-old boy, had a remarkable tendency to form keloid skin scars. Decannulation was achieved in 30 of the 31 cases (97%) after an open procedure.

In 15 infants and children, the cricotracheal resection was performed without a tracheotomy. Four grade III stenoses had no preoperative tracheotomy, and in the remaining 11 cases (grade III and IV stenoses), the tracheostoma was resected with the stenosis during the same operative session. Of this latter group, 6 patients were younger than 2 years and 6 were between 2 and 5 years of age at the time of the surgery. These cases were extubated in the intensive care unit at 5 days (3 cases) and 7 days (8 cases) after the operation (Fig 3). Three other cases needed a temporary reintubation after a first failed extubation, but none needed a new tracheotomy. The last patient, a 12-year-old girl with a preoperative grade IV stenosis, was extubated immediately after the operation.

Five patients presented with an associated interarytenoid fixation, which necessitated a combined cricotracheal resection with a midline thyrotomy for the removal of scar tissue and mucosal grafting of

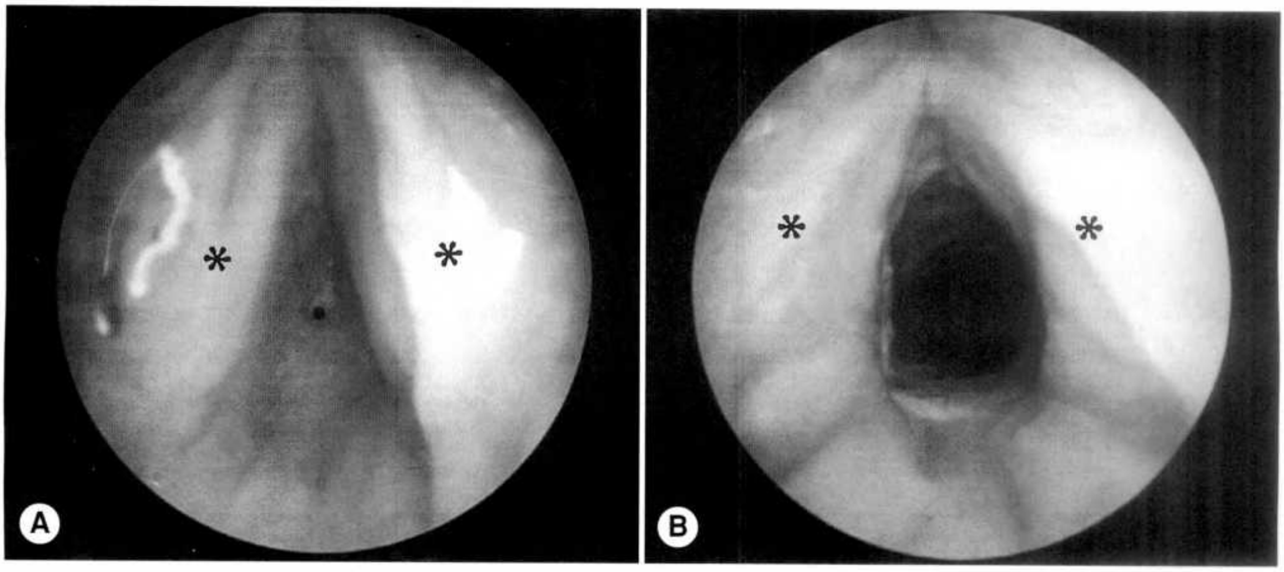

Fig 3. Preoperative and postoperative views of grade III subglottic stenosis acquired after prolonged intubation. Asterisks — vocal cords. A) Near-total subglottic obstruction situated very close to inferior border of vocal cords. B) Three months postoperatively, subglottic lumen is widely patent. Subglottic anastomosis is situated immediately underneath vocal cords. Clinically, voice and respiration are normal.

the posterior commissure as described in Materials and Methods. Four of these 5 cases were stented with a homemade rubber-silicone laryngotracheal prosthesis for at least 6 weeks. One patient was kept intubated for 10 days and then extubated uneventfully.

It is worthwhile to note that we experienced no injury to the RLNs and no mortality in this series of 31 cases.

Twenty-seven of the 31 patients engage in sports freely without exertional dyspnea. Three patients live fully normally, but present a slight stridor with some dyspnea while exercising. One patient is still tracheotomy-dependent (Table 2).

The postoperative evaluation of the voice was only the subjective report of parents and physicians. In 21 cases, the voice is normal. This represents mainly the group of pure SGS. Nine patients have a moderate dysphonia or a change in the pitch of their voice, probably due to the resection of the cricothyroid muscles. Among this latter group, 5 had an associated laryngofissure for the removal of a cicatricial interarytenoid fixation. Some patients presented with a laryngeal configuration that prevented the postoperative result from being optimal (Fig 4). The subjective reports of preoperative and postoperative voice quality are shown in Table 3.

TABLE 2. POSTOPERATIVE DATA ON 31 PEDIATRIC CRICOTRACHEAL RESECTIONS

Postoperative Data	*No. of Cases*
Mortality	0
Recurrent nerve lesion	0
Complete restenosis	1
Decannulated	30
Slight exertional dyspnea	3
No exertional dyspnea	27

Postoperative follow-up is longer than 10 years in 8 cases, 5 to 10 years in 6 cases, and 1 to 5 years in 12 cases. Four patients have reached adulthood; the longest follow-up is now 18 years. Based on the absence of symptoms in 12 patients and on endoscopy in 10 others who were followed up for more than 5 years, the laryngotracheal growth is normal.

Complications. Although we had no case with a lesion of the RLNs and no mortality, there was 1 complete failure in a grade IV glotto-subglottic stenosis. This was in a 13-year-old boy who experienced a complete restenosis of the airway.

Another major complication, not related to the surgery itself but probably influenced by it, occurred on the fifth postoperative day in a 12-year-old girl, immediately extubated after the cricotracheal resection. This girl never experienced any dyspnea during the first postoperative days. In fact, she did so well that she was allowed to walk around her room. While walking, all of a sudden, she complained of a weakness in her legs followed by a difficulty in breathing, without stridor. The child was reintubated and immediate revision surgery showed an intact anastomosis with a normal lumen. The final diagnosis, made by magnetic resonance imaging, was an ischemia of the upper thoracic medulla with subsequent paraplegia. It must be mentioned that the initial trauma that necessitated the prolonged intubation respon-

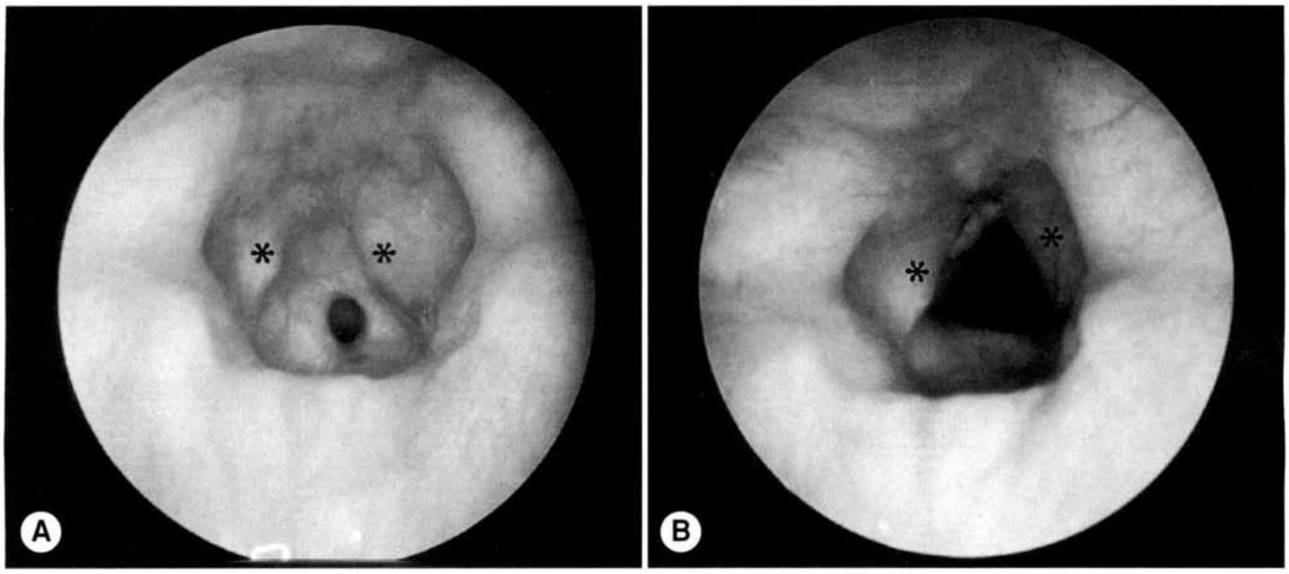

Fig 4. Preoperative and postoperative views of recurrent grade III subglottic stenosis after failed laryngotracheoplasty. Asterisks — vocal cords. A) Preoperative view. Subglottic stenosis reaching inferior border of true vocal cords. Notice widening of anterior commissure resulting from cartilage insert; false vocal cords are completely separated aside. B) Postoperative view at 10 months. Widely patent subglottic lumen with better approximation of true and false vocal cords at anterior commissure of larynx. Clinically, there was no exertional dyspnea, but there was residual dysphonia.

sible for the SGS had already induced a hemiplegia from which the patient had recovered completely before the present surgery. The exact mechanism of this severe complication remains unclear, but it was probably vascular in origin.

Two other patients who presented with a partial dehiscence of the anastomosis needed a second open procedure and had an excellent final result. The first was a 4-year-old boy who was inadvertently extubated during the third postoperative night in the intensive care unit. He was traumatically reintubated by an inexperienced resident. This resulted in a partial anterior dehiscence of the anastomosis that was successfully repaired a few hours later. The child made an uneventful recovery and was extubated after 10 days. The final result has been optimal, with a 90% lumen at the site of the anastomosis.

The second patient was a 2-year-old girl with previously failed laryngotracheoplasty who needed the resection of the subglottis plus 6 tracheal rings to solve her problem. The operation was carried out without laryngeal release. The left partial dehiscence of the anastomosis was repaired at 10 days by using 3 more stitches to bring the thyroid cartilage and the tracheal ring together. The right two thirds of the anastomosis was intact. The final result shows a large subglottic lumen with a slight exuberant scar at the left anterolateral aspect of the anastomosis (Fig 4).

One other minor complication occurred that was due to an anterior granulation partially obstructing the subglottic airway. This was easily treated by endoscopic removal with the carbon dioxide laser. The final result was a subglottic airway of normal size.

TABLE 3. VOICE QUALITY IN 31 PEDIATRIC SUBGLOTTIC RESECTIONS WITH PRIMARY TRACHEAL ANASTOMOSIS

Voice	*Preoperative*	*Postoperative*
Normal	4	21
Dysphonia	3	9
Absent	24	1

DISCUSSION

Until now, the reluctance to use a cricotracheal resection to resolve SGS in infants and children has been mainly because of the risk of injury to the RLNs, the possible dehiscence of the anastomosis with subsequent restenosis, and the interference with normal laryngotracheal growth. Further, a cricotracheal resection may seem a more complex procedure than a laryngotracheoplasty.[1,2,4,5,7]

This series of 31 consecutive cricotracheal resections for severe subglottic or glotto-subglottic stenoses in pediatric patients compares favorably with the laryngotracheoplasty procedures usually performed for these indications.

The reasons for this high rate (97%) of success lie in the following: 1) complete resection of the stenosis with restoration of a large lumen using a normal tracheal ring; 2) preservation of normal laryngotracheal girders (no disruption of the cartilaginous framework); 3) full mucosal lining on both sides of the anastomosis, thus preventing granulation tissue formation and restenosis; 4) avoidance of stenting in pure SGS; and 5) normal growth of the laryngotracheal airway until adulthood.

To achieve good results, some recommendations are worth mentioning. First, the tracheotomy needed to bypass an SGS should be performed as close as possible to the stenotic area. This will allow the easy resection of the tracheostoma with the SGS in 1 segment while leaving enough trachea for performing the anastomosis without tension. The same holds true for single-stage laryngotracheoplasties. Second, the tracheal ring used for the anastomosis should be normal and steady to prevent any localized tracheomalacia at the site of the anastomosis. Finally, all stitches should be placed submucosally to avoid granulation tissue on the suture line. Only three 6.0 Vicryl sutures are tied posteriorly inside the lumen to obtain the best approximation of the mucosa under direct vision.

However, the potential complications of the surgery should not be underestimated. The first is injury to the RLNs. With the technique described in this paper, in which the RLNs are not identified but are left aside behind the cricothyroid joints by dissecting the anterior cricoid arch in a subperichondrial plane, the risk of injury is minimal, even in reoperations in which the nerves are embedded in scar tissue.[12,14] While performing the anastomosis, one should, however, be very careful not to tie one RLN with a stitch at the posterolateral edge of the cricoid plate. This is best achieved by placing the stitches in a subperichondrial plane, since the RLNs run in the musculature situated externally to the perichondrium. In the present series, none of the 31 patients have had any injury to the RLNs postoperatively.

The second potential complication is partial dehiscence of the anastomosis. This complication occurred twice (7%) in this series of 31 cases. One instance was definitely due to an excess of tension at the site of the anastomosis. This resulted from the resection of 6 tracheal rings with the SGS. Revision surgery showed that the sutures had progressively cut the inferior edge of the thyroid cartilage. The second case was due to a forceful reintubation in an emergency situation by an inexperienced resident. The key to success lies in avoidance of surgery in the presence of the slightest upper or lower airway infection and in the performance of a laryngeal release when mobilization of the trachea is difficult or tracheal resection is extensive (>5 tracheal rings).[16-18] Most of the cases of dehiscence described in the literature on adults are due to excessive tension on the suture line or to surgery performed in the presence of an infected airway. In children, the position of the larynx high in the neck and the elasticity of the tissues allow an easy mobilization of the trachea. Resection of 5 tracheal rings can be performed without a hilar mobilization or laryngeal release. The absence of calcified tracheal rings in children is very favorable for placing the Vicryl sutures through the cartilage, to emerge submucosally at the edge of the anastomosis. There should not be any visible thread at the site of the anastomosis during the first postoperative endoscopy, except posteriorly. The risk of restenosis always results from an inadequate technique with partial dehiscence of the anastomosis, granulation tissue formation, and subsequent scarring.

The third concern is interference with normal laryngotracheal growth. However, the concern that resecting the anterior arch of the cricoid cartilage in a small child may interfere with subsequent normal subglottic growth is now obsolete. Fearon and McMillin[19] published an experimental study on developing primates in 1985, showing that a cricotracheal resection with primary tracheal anastomosis is perfectly possible in these animals who correspond closely in size to a small child. Unfortunately, the follow-up period was too short to confirm experimentally that laryngotracheal growth is fully normal after this type of operation. The experience gathered in this series of 31 patients, 8 of whom were followed up for more than 10 years, and 4 until adulthood, gives further support to the innocuousness of this procedure in terms of laryngotracheal development. If the initial result of the anastomosis is good, then the thyroid cartilage and tracheal ring sutured together will continue to grow normally.

Finally, there is the potential for worsening of voice quality. In cases of pure SGS, the risk of deterioration of the voice is almost nil, except in cases of lesion to the RLNs. The subglottic anastomosis is situated 2 to 3 mm below the glottis and thus does not interfere with voice production. A change in the pitch of the voice may be expected, since the cricothyroid muscles are resected during the operation. In glotto-subglottic stenosis, the postoperative quality of the voice depends mostly on the preoperative scarring of the vocal cords. Per se, a cricotracheal resection with thyrotracheal anastomosis should not deteriorate the quality of the voice, since the anterior commissure of the larynx is maintained in its initial position.

CONCLUSION

This update of the Lausanne experience, which now includes 31 cases of pediatric severe SGS treated by partial cricoid resection with primary thyrotracheal anastomosis, confirms the outstanding results published in 1993 on the first 15 cases. The decannulation rate is 97% (30 of 31 cases).

The surgical procedure can be combined with resection of an anterior synechia of the vocal cords or with treatment of a posterior glottic stenosis. In these cases, a laryngotracheal stent is needed for 6 weeks before decannulation is possible. This cricotracheal resection is not reserved for pure SGS, but may also play a significant role in glotto-subglottic stenoses.

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PAROTID GLAND CARCINOMA: ANALYSIS OF PROGNOSTIC FACTORS

CARLO CALEARO, MD
FERRARA, ITALY

ANTONIO PASTORE, MD
FERRARA, ITALY

OMERO FINI STORCHI, MD
FLORENCE, ITALY

GIANNI POLLI, MD
FLORENCE, ITALY

The histologic variety of parotid gland carcinomas, their different natural history, and the peculiar anatomy of the parotid region can make prognosis and therapeutic strategy quite controversial. The present study was designed to evaluate those prognostic factors able to affect the long-term results in a group of 167 consecutively treated parotid epithelial malignancies. The continuous or discrete covariants considered as potential prognostic factors are age, sex, histotype, grading, TNM and pTNM classification, facial nerve involvement, type of surgery on the tumor site and on nodes, facial nerve resection, and postoperative radiotherapy. All the material has been statistically analyzed and the results have been compared with the principal data published. According to the analysis, the most relevant prognostic factors in parotid gland carcinomas appear to be pTN staging, tumor grading, facial nerve involvement, and local extension. These factors could reliably predict the patient's chance for survival, and thus influence the therapeutic strategy.

KEY WORDS — parotid gland carcinoma, prognosis, statistical analysis.

INTRODUCTION

Primary parotid gland epithelial malignancies are rare, accounting for approximately 1% to 3% of all head and neck carcinomas. The histologic variety of such tumors, their different natural history, and the peculiar anatomy of the region can make diagnosis, prognosis, and therapeutic strategy quite controversial. Appropriate parotid gland carcinoma management requires up-to-date information regarding those prognostic factors able to affect local, regional, or distant recurrences and survival. Herein, the results of an analysis of the prognostic factors for parotid carcinomas are described based on personal data and reports published on this topic.

MATERIALS AND METHODS

One hundred sixty-seven malignant epithelial neoplasms were examined that had been treated for the first time in the otorhinolaryngology clinics at the Universities of Ferrara and Florence between 1970 and 1990. The study population was composed of 92 male and 75 female patients with ages ranging from 11 to 87 years (mean 57.07 years). The histologic definition is given in Table 1, where one can see that adenoid cystic carcinoma is the best-represented group (24.55%).

TABLE 1. HISTOLOGIC CLASSIFICATION OF 167 NEOPLASMS

	No. of Cases	*%*
Adenoid cystic carcinoma	41	24.55
Mucoepidermoid carcinoma	32	19.16
Adenocarcinoma	29	17.37
Acinic cell carcinoma	22	13.17
Squamous carcinoma	23	13.78
Mixed malignant tumor	10	5.99
Other	10	5.99

On physical examination, 31 cases (18.56%) presented clinically evident regional lymph nodes. The UICC 1987 dimensional classification made it possible to distinguish 37 T1 (22.15%), 81 T2 (48.50%), 35 T3 (20.95%), and 14 T4 (8.38%) tumors (Table 2). Evaluation of the "absence" or "presence" of local extensions (index "a" and "b" according to UICC 1987) revealed 77 cases with local extension (46.11%) and 90 without. In particular, by differentiating extension by either facial nerve involvement, spread to soft tissues and/or bone, or both, 3 sub-

TABLE 2. TN CLASSIFICATION ACCORDING TO UICC 1987

	N0	*N1*	*N2b*	*N3*	*Total*
T1	34	3	0	0	37
T2	66	9	5	1	81
T3	29	5	1	0	35
T4	7	4	3	0	14
Total	136	21	9	1	167

From the Otorhinolaryngology Clinic, University of Ferrara, Ferrara (Calearo, Pastore), and the Otorhinolaryngology Clinic, University of Florence, Florence (Fini Storchi, Polli), Italy.

CORRESPONDENCE — Carlo Calearo, MD, ENT Clinic, University of Ferrara, S. Anna Hospital, Ferrara, Italy.

TABLE 3. RECURRENCES ACCORDING TO HISTOLOGY

	No.	*%*
Adenoid cystic carcinoma	10/41	24.39
Mucoepidermoid carcinoma	1/32	3.13
Adenocarcinoma	8/29	27.59
Acinic cell carcinoma	4/22	18.18
Squamous carcinoma	10/23	43.48
Mixed malignant tumor	2/10	20.00
Other	3/10	30.00

classes were obtained of 36, 27, and 14 patients, respectively. Total parotidectomy was performed in all cases. The surgery was extended to extraparotid structures and sites in 33 subjects (19.76%); because of the clinical evidence of anatomic and functional involvement, 1 or more terminal branches of the facial nerve were resected in 61 patients (36.52%). Total parotidectomy was associated with ipsilateral neck dissection in 47 patients (28.14%): 15 functional (8.98%) and 32 classic radical neck dissections (19.16%).

Postoperative radiotherapy was performed in 68 cases (40.71%): 32 on the T site and 36 on both T and N sites.

The follow-up has been updated to December 30, 1995. All the material has been statistically analyzed with univariate analysis by means of χ^2 and Student's *t*-test for discrete and continuous variables, respectively; multivariate analysis by using the calculation of multiple dependent-variable regressions; and discriminant analysis of covariants.

RESULTS

The event determining therapy failure is most notably the appearance of local, regional, and/or distant recurrences. Therefore, it seems reasonable to statistically assess prognostic factors by considering the recurrence as the end point. The continuous or discrete covariants considered as prognostic factors were age, sex, histotype, grading, TNM and pTNM classification, clinical facial nerve involvement, type of parotid surgery, type of lymph node surgery, facial nerve resection, and postoperative radiotherapy.

There were 38 (22.75%) cases of recurrence in the present study group.

The univariate statistical analysis of the single covariants in relation to the variable "recurrence" showed that the relationship between age and recurrence was statistically significant ($p < .05$). In all classes (N1-3), the presence of nodes proved to be a significant prognostic index ($p < .05$). The ratio between the event "recurrence" and the covariants T and pT stages ($p < .01$ and $p < .001$, respectively) was highly significant, as was the covariant "local extension" ($p < .001$). The type of surgery is related to recurrence, since the need for extended surgery is statistically and prognostically unfavorable ($p < .05$). No other covariant showed a significant relationship to the dependent variable "recurrence."

A separate analysis is in order for the covariant "histotype" (Table 3). A simple descriptive study stresses the differences in the incidence of recurrence by histotype. In the present sample, recurrence was rarely encountered in the mucoepidermoid histotype, but was progressively more apparent in the other histotypes, peaking for squamous carcinoma (43.48%). However, this covariant was not statistically significant for any histotype except mucoepidermoid carcinoma ($p < .05$). Finally, it is worth noting that complementary radiotherapy to the primary tumor and/or nodes (68 of 167 patients) did not favorably affect prognosis for the dependent variable "recurrence."

A "1-way" variance analysis was also employed within all covariants. The only significant finding was a highly significant correlation between nodes and local spread of tumor and between tumor stage and facial nerve involvement.

Subsequently, a multivariate analysis was employed using multiple regression to study "recurrence" as a dependent variable. The study demonstrated that the factors considered could account for 33% of the recurrences, while the remaining 67% stemmed from other, untested factors.

Among covariants, the greatest importance must be ascribed to the pTN staging. No major prognostic factor seems to be present among those considered.

The discriminant analysis of the most significant covariants for the above-mentioned tests (age, sex, histotype, T and N staging, facial nerve involvement, local spread, pT and pN staging) demonstrates that 87.5% of the unrecurrent cases were prognosticated as such and that 77.8% of the recurrent cases could also be predicted. The statistical specificity index obtained with the present method is 85.8%. This confirms that these initial factors may constitute a good prognostic model to start working with.

DISCUSSION

Histology. Histopathologic tumor type appears to be a relevant prognostic factor, particularly in terms of different biologic tumor behaviors.[1-9] Although there is no absolute agreement between the various authors, it is evident that there are different "degrees" of malignancy. In this light, undifferentiated, squamous, malignant mixed, adenoid cystic carcinomas, and adenocarcinoma have the worst prognosis, while mucoepidermoid and acinic cell carcinomas have the best. Because of the slow tumor kinetics of some histotypes (adenoid cystic and acinic cell carcinomas), this is especially true when follow-up is in excess of 10 years.[1] According to Spiro et al,[8] about 85% of all patients with acinic cell carcinoma are alive at 10 years, whereas this is true for only about 44% to 50% of those with malignant mixed tumor, adenocarcinoma, or adenoid cystic carcinoma. Patients with squamous carcinoma have survival rates of less than 30% at 10 years. Mucoepidermoid tumor survival rates appear strictly linked to tumor grading; thus, low-grade tumors present 10-year survival rates of up to 85%, while intermediate or high-grade lesions have a rate of only 45%.

Bjorklund and Eneroth[1] confirm a 10-year survival rate of approximately 85% for mucoepidermoid and acinic cell carcinomas, while the worst prognosis, down to less than 25% at 15 years, is found with poorly differentiated solid carcinomas, malignant mixed tumors, and adenoid cystic carcinoma. The same authors stress the fact that for acinic cell carcinoma, and above all for adenoid cystic carcinoma, prognosis cannot be based on a follow-up of less than 10 to 15 years.

Kane et al[10] report a 5-year survival rate of more than 90% in low-grade mucoepidermoid carcinoma and of about 80% in the acinic cell and adenoid cystic carcinomas. They also found a dramatic drop in the survival rate from 5 to 15 years in the adenoid cystic and acinic cell carcinomas, while the figures for mucoepidermoid carcinoma appeared to change only slightly during the course of an extended follow-up. The same authors stress the fact that adenoid cystic and acinic cell carcinomas are the only histotypes capable of causing death after 10 years disease-free. In their experience, high-grade mucoepidermoid and squamous carcinomas have intermediate survival rates (approximately 40% at 10 years), whereas for all the other carcinomas, the prognosis is poor.

Pedersen et al[7] note that the longest recurrence time is found in cases of acinic cell carcinoma, followed by mucoepidermoid and adenoid cystic carcinomas; the shortest time to recurrence (10 months) was found in patients with malignant mixed tumors. According to these authors, 10-year survival rates are very high, up to 86% for acinic cell tumors, declining to 35% for malignant mixed tumors. Adenocarcinoma and mucoepidermoid, adenoid cystic, and undifferentiated carcinomas show survival rates from 55% to 44%. The worst prognosis was found in the malignant mixed tumor group.

Calearo et al,[2] in their therapeutic planning for malignant epithelial tumors, recognized different classes of local or regional risk of recurrence; thus, a better prognosis is recognized for mucoepidermoid and acinic cell tumors. Adenoid cystic carcinoma has a good 5-year follow-up prognosis, which worsens after 10 to 15 years of follow-up. All the other carcinomas show poor results within the first 3 years.

This present study is in complete accordance with those previous data.

Local Extension, T Category, Stage, and Grade. A covariant that proves strongly related to prognosis is local extension.[7] According to our data, this is particularly true for patients with T1 and T2 tumors. In these tumors, when locally extended, survival rates drop to less than 50% of those in tumors of the same stages but without local extension.

Facial nerve involvement is an extremely important prognostic index for both recurrence and survival, since very few patients presenting facial paralysis survive their disease.[7,8,11-16] In a study on 378 malignant parotid tumors, Eneroth and Hamberger[3] found 100% mortality in patients with facial nerve paralysis. The same was observed by Pedersen et al.[7] According to all the authors reviewed, survival for patients with spontaneous facial nerve palsy is at most between 10% and 30%. In our experience, clinical facial nerve involvement is highly significant ($p < .001$) for both recurrence and final prognosis, while microscopic surgical involvement is not ($p > .5$).

In all the studies in which the covariants T and pT stage were present, these appeared highly related to both recurrence and disease-free survival.[9,14,16-19] Similarly, the presence of local-regional adenopathies proves highly significant for a poor prognosis.[8,14,16] According to Spiro et al,[8] stage and nodal status at diagnosis are the most significant prognostic variables in a Cox regression analysis.

Frankenthaler et al[14] assert that positive cervical nodes, tumor size greater than 3 cm, and nerve invasion are the factors having the greatest effect on survival rate. Indeed, when these factors were all present at the same time, the risk of dying of disease was 67 times greater than that for the study population as a whole. Our personal data revealed a highly significant correlation between recurrence and T stage ($p < .01$), pT stage ($p < .001$), and N classification ($p < .05$).

Only in the case of the highest grade does tumor grading appear significant in the prognosis of both recurrence and survival.[6,7,14,20,21] Kane et al[10] consider grading the most reliable prognostic index, especially for mucoepidermoid carcinoma. Conley and Hamaker[11] claim this also holds true for adenocarcinomas and acinic cell tumors. While Spiro et al[8] confirm that grading can provide some prognostic information for mucoepidermoid or acinic cell carcinoma, they found no reliable correlation for adenoid cystic carcinoma. Further, they were unable to find any correlation between grading and survival. Some authors[10,19] found that grade and stage had a significant correlation with survival, while others did not.[6] Grade and tumor size have also been considered key factors in predicting distant metastases.[14]

Therapy. In a retrospective study it is always difficult to assess the effect different therapeutic approaches have, although the impact therapy has on prognosis does appear obvious. To our knowledge, no randomized study exists comparing surgical treatment alone with combined therapy (surgery plus radiotherapy). Nevertheless, there is evidence in the literature indicating the need to review treatment strategies. Many authors consider radiotherapy very important in preventing local-regional recurrences[6,14,17,21-23] and improving survival.[23-25] On the other hand, while admitting the role combined therapy can play, other authors feel, as we do, that its specific use has still to be codified.[2,7]

On a large series of patients treated before and after 1965, Spiro et al[8] were unable to demonstrate that use of combined therapy translated into any real improvement in survival; this may, however, be due to the fact that the patients receiving irradiation were always those with more extensive tumors and thus the most unfavorable prognosis. In contrast, Shidnia et al[18] assert that combined treatment can improve survival in all patients except those with low-grade mucoepidermoid and early acinic cell carcinomas. Matsuba et al[6] report a significant improvement in local-regional control with combined therapy, although they did not achieve improved survival rates using this therapeutic protocol. Several authors advocate radical surgery, comprising routine facial nerve sacrifice, in all high-grade malignancies.[4,26,27] On the other hand, many others failed to find evidence that survival improved as a result of more radical surgery.[9,17] Most authors today consider the best therapeutic strategy to be conservative surgery followed, when indicated, by postoperative irradiation.[2,6,9,14,16,24] In this light, radiotherapy appears to play a prognostically significant role in the postsurgical approach to patients with stage III or IV tumors, high-grade lesions, or recurrent carcinomas, and whenever there is microscopic or macroscopic facial nerve involvement.

Age, Sex, and Symptoms. Age has been considered a prognostic factor by some other authors[14] and by us; this may be explained by the fact that older patients tend to have a higher percentage of high-grade and advanced-stage lesions.[8] Male sex has been identified as an independent prognostic factor,[10] while other authors[8] rule gender out of the prognostic index. Prognostic significance has been attributed to pain, paresthesia, and rapid enlargement at the time of presentation,[10,17,26] although there is no general agreement on these topics.

CONCLUSIONS

The most relevant prognostic factors in parotid gland carcinomas appear to be pTN staging, tumor grading, facial nerve involvement, and local extension. These factors, along with other information (age, sex, symptoms, delay of diagnosis, etc), should always be submitted to statistical analysis in order to reliably predict the patient's chances for survival.

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DYNAMICS OF SUBNEOGLOTTIC LUMEN IN TRACHEOESOPHAGEAL PHONATION

MITSUHIRO MOHRI, MD
MINORU KINISHI, MD
MUTSUO AMATSU, MD
KOBE, JAPAN

The patient who employs tracheoesophageal (TE) phonation uses expiratory air passing through the TE fistula to vibrate the mucosa of the neoglottis located in the hypopharynx. To clarify the dynamics of the subneoglottic lumen during phonation, we performed fiberoptic endoscopy and fluoroscopy in 13 TE speakers. During phonation, fiberoptic endoscopy showed concentric closure of the esophagus with ballooning of the subneoglottic lumen, followed by opening of the esophagus during inspiration. Fluoroscopy revealed a dilated subneoglottic lumen and a closure of the esophagus at the bottom of the lumen during phonation. These findings provide evidence for a mechanism to protect against the influx of air deep into the esophagus during TE phonation. We conclude that during TE phonation, a closed airway is established from the lung to the neoglottis that enables the TE speaker to use expiratory air effectively for phonation.

KEY WORDS — esophageal closure, laryngectomy, subneoglottic lumen, tracheoesophageal phonation, voice restoration.

INTRODUCTION

Voice rehabilitation is required for laryngectomy patients to maintain their quality of life. The currently available means of postlaryngectomy voice restoration are esophageal speech, use of an artificial device, and tracheoesophageal (TE) speech with or without a prosthetic device. The TE procedure is considered to be the most effective, since it uses the powerful expiratory air passing through a surgically constructed fistula to vibrate the neoglottis that was formed on the hypopharyngeal mucosa. Since 1976, we have performed TE fistulization as described elsewhere,[1,2] with good results in terms of the intensity of the voice, fluency, and the ease of acquisition of a substitute voice as compared with the conventional esophageal voice.[1,3]

The voice intensity and fluency that characterize TE phonation depend mainly on the force of expiratory air introduced into the subneoglottic lumen through the TE fistula, provided that a closed airway is established without leakage from the lung to the neoglottis. The neoglottis in TE phonation has been the subject of special investigation by many researchers, since it has been considered to play a chief role in the phonatory mechanism.[4-7] Although many studies have been done on the neoglottic activity, little research has been done on the subneoglottic lumen that is composed of the hypopharynx and the upper esophagus.[8,9] The present study was undertaken to clarify and explore the role of the subneoglottic lumen in TE phonation.

SUBJECTS AND METHODS

We evaluated 13 Japanese subjects, 12 men and 1 woman, ranging in age from 47 to 77 years (mean age 62 years). At the time of laryngectomy at Kobe University Hospital, each subject underwent TE fistulization as previously described.[1,2] All subjects were examined over a 6-month postoperative period, once they could converse well without impediment in the TE speech.

The study protocol consisted of fiberoptic observations and fluoroscopy of the hypopharynx and esophagus performed during TE phonation. Subjects were instructed to phonate a sustained vowel /a/ during each examination. Fiberoptic observation was done transnasally, and the subneoglottic lumen was observed through the neoglottis. Fluoroscopy was performed to clarify the configuration of the subneoglottic lumen. The dynamics of the subneoglottic lumen during phonation were evaluated in the lateral projection.

RESULTS

The introduction of a flexible fiberscope into the neoglottis allowed the direct visualization of the dynamics of the subneoglottic lumen that extended from the hypopharynx to the upper esophagus. The common finding during phonation in each subject was a ballooning of the subneoglottic lumen with a concentric closure of the esophagus at the lower end of the lumen that was located caudal to the orifice of

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From the Department of Otorhinolaryngology–Head and Neck Surgery, Kobe University School of Medicine, Kobe, Japan.

CORRESPONDENCE — Mitsuhiro Mohri, MD, Dept of Otorhinolaryngology–Head and Neck Surgery, Kobe University School of Medicine, 7-5-2, Kusunoki-cho, Chuo-ku, Kobe, 650, Japan.

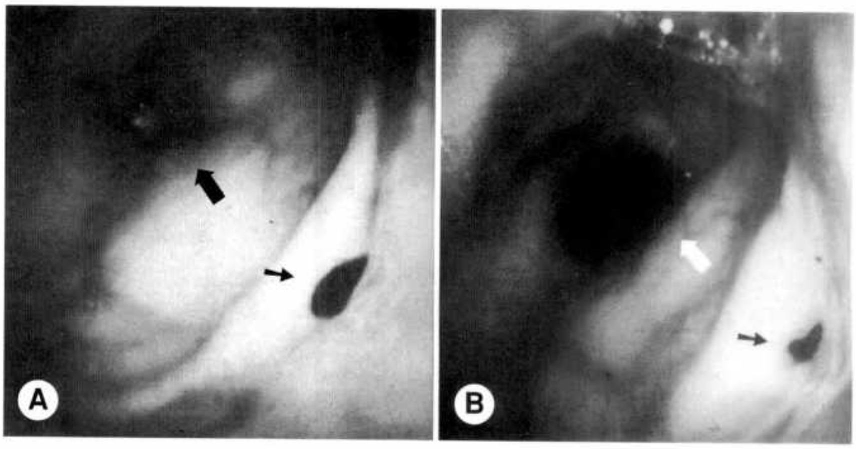

Fig 1. Fiberoptic findings of subneoglottic lumen. A) Closure (large arrow) during phonation. Orifice of tracheoesophageal fistula (small arrow) is enlarged by diverted expiratory air. Closed point of esophagus is distal to orifice. B) Opening (white arrow) after phonation. Orifice of fistula (black arrow) is closed.

the TE fistula (Fig 1A). After phonation, the expanded lumen shrank and the closed esophagus opened for the inspiratory phase (Fig 1B).

Fluoroscopy revealed a dilatation of the subneoglottic lumen with long distal closure of the esophagus during phonation in each subject (Fig 2). The bottom of the dilated subneoglottic lumen was easily distinguished from the closed esophagus.

DISCUSSION

A considerable number of studies have been done on the source of vibration in the mechanism of alaryngeal phonation.[4-7,10-12] Radiographs of the lateral neck have shown a retropharyngeal prominence that protruded into the lumen during esophageal phonation[10-12] and during TE phonation.[4-6] The neoglottis as the vibratory source has been assumed to be located on this prominence. We previously described the contraction of the musculature that composes this prominence and its role in TE phonation.[7] In brief, the thyropharyngeus muscle contracts to narrow the hypopharyngeal lumen before phonation and to maintain a definite configuration of the neoglottis during phonation.

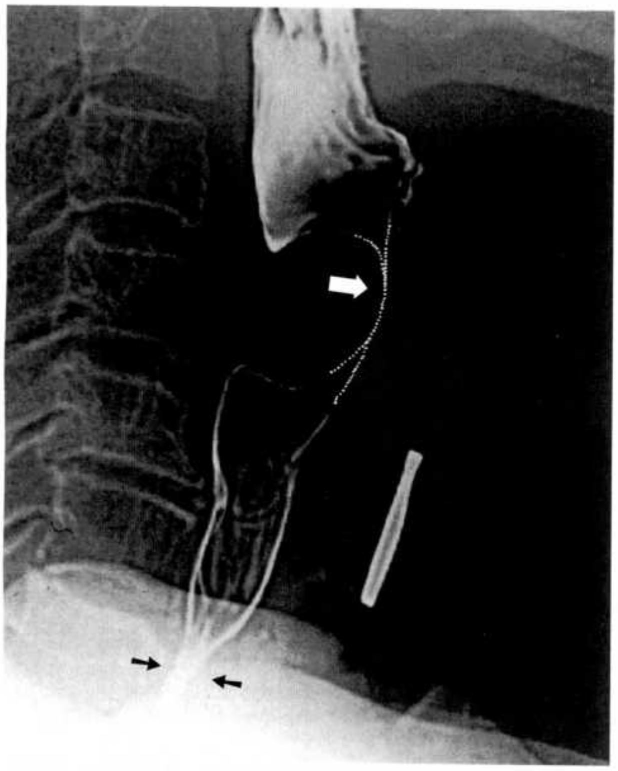

Fig 2. Lateral projection on fluoroscopy. Neoglottis (white arrow) and upper end of esophageal closure (black arrows) are demonstrated. Note dilated subneoglottic lumen.

Less attention has been paid to the dynamics of the subneoglottic lumen during TE phonation. It is generally believed that the exhaled pulmonary air that is diverted into the esophagus can easily rise to vibrate the mucosa of the neoglottis. However, the air can move downward as well as upward, following the line of least resistance. The contraction of the thyropharyngeus muscle for the neoglottic formation that narrows the lumen essentially plays the role of resistance for the ascending airflow. Thus, without some protective mechanism, the diverted air would descend. The ability of TE speakers to speak loudly with good fluency suggests the presence of some mechanism to protect against air influx down into the esophagus.

There are a few reports on the function of the subneoglottic lumen.[8,9] Wolfe et al[8] reported the presence of a hiatal hernia and/or gastroesophageal reflux in patients who failed to attain adequate rehabilitation of esophageal speech. These investigators pointed to the need for a competent distal esophageal sphincter to trap a usable supply of air in the esophagus, and emphasized the significance of some protection against the influx of the air down into the stomach. Tracheoesophageal phonation would also necessitate such a protective mechanism, since it uses even more air than esophageal speech.

In the present study, fiberoptic examination disclosed a concentric closure of the esophagus at the bottom of the ballooned subneoglottic lumen during TE phonation. Fluoroscopy also revealed a dilatation of the subneoglottic lumen with esophageal closure at its bottom during phonation. From these findings, the concentric closure of the esophagus seen on fiberscopy corresponds to the bottom of the dilated subneoglottic lumen, ie, the top of the closed segment of the esophagus observed on fluoroscopy. This closure is thought to protect against the influx of air down into the esophagus. This protective mechanism ensures an upward movement of air in the subneoglottic lumen. Thus, a closed airway from the lung to the neoglottis is established that enables the TE speaker to use pulmonary air for phonation most effectively.

The closure of the esophagus may be regarded as a compensatory adaptation for the loss of the larynx. Alternatively, such closure may be explained as a preserved motility after laryngectomy, on the basis of the assumption that the cervical esophagus is essentially closed during normal laryngeal phonation. Although the control mechanism is unknown, the closure of the esophagus is thought to be regulated by the esophageal branch of the recurrent nerve, which innervates the cervical esophagus and is usually preserved during laryngectomy.

CONCLUSION

The following conclusions were made. First, a mechanism to protect against the influx of the diverted expiratory air down into the esophagus is established during TE phonation. This is achieved by the concentric closure of the esophagus distal to the orifice of the TE fistula. Second, this protective mechanism ensures a closed airway from the lung to the neoglottis during TE phonation and allows the TE speaker to use air effectively for phonation.

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MIDBRAIN REGIONS FOR ELICITING VOCALIZATION BY ELECTRICAL STIMULATION IN ANESTHETIZED DOGS

KANG LIU, MD, PHD
IOWA CITY, IOWA

NANCY PEARL SOLOMON, PHD
MINNEAPOLIS, MINNESOTA

ERICH S. LUSCHEI, PHD
IOWA CITY, IOWA

Eliciting vocalization in anesthetized dogs by midbrain stimulation is a useful procedure for studies of laryngeal and respiratory physiology. The goal of this report has been to construct a canine stereotaxic "map" that would allow investigators to locate midbrain stimulus sites producing vocalization. Motor responses to electrical stimulation at currents of 1.5 mA or less were observed at 1,158 stimulus sites throughout the midbrains of 8 dogs. Vocalization was observed at 213 stimulus sites. The highest probability of observing vocalization was for sites located 6 to 10 mm anterior, 6 to 7 mm lateral, and 5 to 8 mm dorsal to earbar zero. The vocalization region most likely consists of axons arising in the midbrain periaqueductal gray and coursing through the adjacent tegmentum; low-threshold sites are close to the medial lemniscus. The relationship between stimulus sites at which vocalization was elicited and sites producing other motor responses is described.

KEY WORDS — canine, dog, evoked phonation, evoked vocalization, laryngeal physiology.

Eliciting vocalization in anesthetized animals has been a valuable model for studies of laryngeal and respiratory neurophysiology. Vocalization has been elicited by electrical or chemical stimulation of the midbrain in monkeys,[1-4] cats,[4-11] rats,[12] guinea pigs,[13] bats,[14,15] and dogs[16-18] under anesthetized, decerebrate, or awake conditions. Such stimulation has been demonstrated or presumed to excite the midbrain periaqueductal gray (PAG) and the adjacent tissue of the tegmentum.

From previous literature and our own experiences, we have developed a technique for electrical stimulation of the midbrain for evoking vocalization. It was successfully applied in anesthetized cats for investigating the relationships between stimulus parameters (intensity, duration, and frequency) and characteristics of the elicited vocalization and associated activity of laryngeal and respiratory muscles.[10] The dog, however, is the preferred species for our experiments, because of the relatively large size of the canine larynx and the morphologic similarity between canine and human larynges.[19,20] In addition, the canine larynx has been studied extensively regarding anatomy, histology, physiology, and biomechanics.[21-24]

We have conducted a few studies using electrical stimulation to elicit vocalization in the anesthetized dog,[16,17] and have been able to find sites in the midbrain that, when stimulated, produce robust responses. Potential stimulation sites were explored by advancing the stimulating electrode through the midbrain along several tracks. Decisions regarding track placements were guided by previous reports for the monkey[1,4,25] and cat[4,8,9] and an atlas based on the beagle brain.[26] In our laboratory, we use adult mixed-breed dogs rather than pedigreed beagles, primarily because of size, availability, and cost. Because the atlas for the beagle was not necessarily accurate for our experimental animals in terms of absolute dimensions and brain-skull relationships, our technique was less than optimal. We reasoned that a "map" of the different motor responses produced by midbrain stimulation might improve our efficiency in finding the best sites for eliciting vocalization. Thus, the purpose of this study was to develop a description of functional relationships between various motor responses to electrical stimulation of the canine midbrain. The specific aim was to develop a "behavioral-functional map" to define the stereotaxic coordinates of the boundaries of a midbrain region for the elicitation of vocalization. Preferably, such a "vocalization region" would contain sites at which natural-sounding vocalization would

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From the Department of Speech Pathology and Audiology and the National Center for Voice and Speech, The University of Iowa, Iowa City, Iowa. This research was supported by grant P60 DC00976 from the National Institutes of Health/National Institute on Deafness and Other Communication Disorders. Support for Dr Solomon during manuscript preparation was provided by the Department of Communication Disorders at the University of Minnesota. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of The University of Iowa.

CORRESPONDENCE — Erich S. Luschei, PhD, 122B WJSHC, Dept of Speech Pathology and Audiology, The University of Iowa, Iowa City, IA 52242.

be elicited reliably without other, nonvocal, motor responses.

METHOD

Preparation of Experimental Animals. Nine adult mongrel, hound-like dogs, 18 to 22 kg, were sedated initially by intramuscular injection of a mixture of ketamine hydrochloride (7.5 mg/kg) and xylazine (2.5 mg/kg). A catheter was then placed in the femoral vein for administering physiologic saline and anesthetic drugs. A surgical level of anesthesia (muscle relaxation and the absence of corneal and deep pain reflexes) was induced and maintained by intravenous sodium pentobarbital (25 mg/kg) throughout the experiment. Body temperature was maintained at 36°C to 38°C.

With the dog in the prone position, the head was fixed in a stereotaxic frame (model 1430, Kopf Instruments, Tajunga, Calif) with the Kopf dog adapter. This stereotaxic frame could be rotated around its front mounting, thus allowing the head and body to be turned to the supine position. This made it possible to access the larynx after the head had been placed in the stereotaxic frame.

The larynx and trachea were exposed by a midline incision extending from the hyoid bone to the fifth tracheal ring. To monitor tracheal pressure, a small plastic tubing adapter was placed in a small incision in the connective tissue of the trachea between the second and third rings. The plastic tubing adapter was sutured tightly in place and connected, by a 20-cm length of silicone tubing (2.5 mm inside diameter), to a pressure transducer (MicroSwitch 143PC03G). Because the plastic adapter only protruded a few millimeters into the lumen, it did not occlude the trachea. (Note that the diameter of the trachea in these dogs was about 1.5 to 2 cm.) A custom-designed brace for providing a clear view of the larynx was inserted into the mouth to open the jaw, anchor the tongue, and fix the epiglottis. The animal then was rotated back to a prone position, the scalp was incised along the midline, and the temporalis muscle was retracted laterally to expose the dorsal surface of the skull. A large round dental bur was used to open a 1.5- to 2-cm hole in the skull at the appropriate location (depending on the approach to be used with the electrode; see below). After cutting and retraction of the dura mater, a monopolar microelectrode held in a stereotaxic carrier was inserted into the brain through the cortex to deliver the electrical stimulus. The microelectrode was made from an epoxy-insulated tungsten wire, 0.2 mm in diameter, that had been electrolytically etched to a very fine point. The electrode shaft was stiffened by ensheathing it in a stainless steel tube, starting about 1 cm above the tip of the electrode. The tip of the electrode was uninsulated for 0.1 mm. The indifferent electrode (anode) for electrical stimuli was a large needle placed under the skin over the shoulder.

The reference point of the anterior-posterior (AP), medial-lateral (ML), and dorsal-ventral (DV) axes of the coordinate system was the midpoint between the 2 earbars inserted in the bony ear canals of the temporal bone. This point was defined as zero on all axes, and will henceforth be called "earbar zero." Every stimulus site was described by its AP, ML, and DV distances in millimeters relative to earbar zero. Positive AP, ML, and DV coordinates mean that the stimulus site was anterior, medial, and dorsal to earbar zero, respectively. The anatomic locations of the electrode tip in the midbrain were estimated according to the atlas of the canine (beagle) brain.[26] Electrical stimuli consisted of trains of 0.2-millisecond rectangular monophasic constant-current pulses, with the electrode as cathode, delivered at a rate of 200 Hz. The train of stimuli was usually 2 seconds, but was sometimes extended up to 4 seconds. The current level varied, but generally was between 0.02 and 1.5 mA.

In the first 4 dogs used in the series, the electrode was advanced in a vertical orientation. On later inspection of the specimens, blood was observed in the tracks created by the passage of the electrode through the midbrain. The bleeding probably resulted from damage to the vascular plexus covering the tectum of the brain. In the next 2 animals, the electrode was tilted 30° anterior to the coronal plane. In an additional dog, from which no data were collected, the same approach was used and the electrode tip hit the bony tentorium, was bent, and produced a great deal of damage. In the last 2 animals, the electrode was tilted 14° lateral to the sagittal plane; this approach appeared to be best in terms of minimizing damage to the brain. In data analysis, these angular electrode penetrations have been used to trigonometrically calculate the true stereotaxic coordinates of the stimulus sites.

Experimental Protocol. All 9 dogs received unilateral stimulation of the midbrain: 7 in the left side and 2 in the right side of the midbrain. The exploration was done with the animal in the prone position and commenced with a track located at AP 8, ML 6 (ML 5 in dog 3), then extended track by track with 2-mm (1-mm in dog 3) intervals between tracks. In each track, the electrode was advanced ventrally from DV 20 to DV 5 (DV 3 in some tracks) in 1-mm steps. At each of these sites, electrical stimuli were delivered at or shortly after the end of the inspiratory phase of a respiratory cycle. The interval between successive stimulus trains was 15 to 20 seconds. The delivered current typically started at 1.5 mA and was decreased by 0.1-mA steps, if a response occurred, until no response was elicited. At some sites, the elicited movements of the jaw or body were so strong that the current had to be limited to relatively low levels (eg, below 0.5 mA). In every dog, the explored areas in the midbrain included a clear vocalization region. Once an electrode track was found that evoked vocalization, tracks in every direction from that "vocalization" track were explored until the vocalization was no longer observed at any depth in the track.

After the final stimulation, the electrode was returned to the site at which the "best" vocalization (a clear, loud voice with little or no other motor response) had been elicited. The previously observed responses were always repeated, although in some cases the electrode had to be moved up or down 0.5 to 1 mm. A lesion was made at this best-vocalization site by delivering a 1-mA DC current for 20 seconds. Then, the dog was deeply anesthetized and perfused with saline followed by 10% formalin solution. The brain was postfixed in 10% formalin for at least 1 week. The midbrain was later sectioned to examine the anatomic locations of the electrode tracks and the lesion. Unfortunately, technical problems with the histologic method used precluded detailed study of the tissue, in terms of exactly identifying stimulus sites.

Data Recording. Signals representing the duration and intensity of the stimulation, tracheal pressure, and sound (transduced by a microphone fixed on a maxillary canine tooth), were recorded on an FM tape recorder (HP 3968A, band pass DC 2.5 kHz). These signals were displayed simultaneously on a laboratory computer with data acquisition software (DATAQ Instruments Inc, Akron, Ohio). After the experiment, these signals were low-pass filtered at 500 Hz and then digitized at a sampling rate of 1 kHz per channel. The movement of the larynx was visualized by a video camera focused on the vocal folds through the mouth and displayed on a color monitor. The video image of laryngeal movements occasionally was recorded on videotape.

All observed responses (vocalization, vocal fold movement, movements of body parts, and respiratory activity) and stimulation conditions were recorded in a notebook. Two experimenters provided observations for every stimulation; one attended to the monitor that displayed the view of the larynx, and the other focused on the face and body of the dog. An evoked response was defined as vocalization or any other movement during or immediately following relatively low-intensity stimulation. Vocalization was recognized by both experimenters as a laryngeally generated quasiperiodic sound. Stimulation trials were repeated until the experimenters were confident of their observations. For the purposes of this report, notes made by the observers, rather than the physiologic measures, were the primary source of information.

Data Analysis. The stereotaxic coordinates, stimulus current, and characteristic response of each site at which stimulation produced any observable response were entered in a spreadsheet. Although several trials involving various stimulus currents were conducted at each stimulus site, the current chosen for data entry was approximately 1.5 times the threshold response and did not exceed 1.5 mA. At threshold, there was almost always just 1 very weak response. At 1.5 times threshold, the response was definitive. Response types were categorized as "voice," "face," "body," "jaw," or some combination of these. A few other types of response were also observed (see below). Specific responses included in each category are described in Results.

Stimulus site location was calculated trigonometrically from the electrode carrier and stereotaxic frame readings, and was based on the angle of electrode insertion. The resulting values for each dimension (in millimeters) of the coordinate system were rounded to the nearest integer. Data then were pooled into small regions or "bins" (1 mm for the DV and ML dimensions and 2 mm for the AP dimension). The probability of observing certain types of responses was calculated for each of these small regions. This was accomplished by constructing response frequency histograms of the AP, ML, and DV coordinates of the stimulation sites.

Response frequency histograms were constructed for each of the 4 basic response types alone ("only" groups; Table 1) and for another 4 groups, designated the "plus" groups. "Plus" groups were formed by combining all groups containing the designated type of response (body, face, jaw, voice) in combination with any of the other type(s) of response. For example, the "face plus" group was formed from the combination of rows 5, 8, 9, 11, 13, 14, and 15 in Table 1. In this example, the "face only" group had 261 observations, and "face plus" had 442 observations. It should be noted that the rows in Table 1 are exclusive, so a stimulus site could only be counted once in any of these groups. It should also be noted that the "only" group of a response type is exclusive of the "plus" group of that response type.

Frequency distributions of the coordinates of the

TABLE 1. FREQUENCY OF OBSERVATION OF VARIOUS RESPONSE TYPES, AND ALL THEIR POSSIBLE COMBINATIONS

Table Row No.	Response	No. of Observations
1	B only	91
2	F only	261
3	J only	110
4	V only	76
5	B&F	141
6	B&J	70
7	B&V	8
8	F&J	82
9	F&V	58
10	J&V	9
11	B&F&J	102
12	B&J&V	3
13	F&J&V	8
14	B&F&V	38
15	B&F&J&V	13
	Total	1,070

B — body, F — face, J — jaw, V — voice. Table row number is included for reference to example presented in text.

entire data set are shown in Table 2. The "total sites" column represents the total number of sites stimulated at various coordinates irrespective of whether a response was observed. The cells of the "total responses" column represent the number of sites at which any response was elicited at currents of 1.5 mA or less. There were a total of 1,158 of these sites. Other columns represent the number of times various types of responses occurred. The difference between the "total sites" and the "total responses" is largely dependent on the inclusion of sites that were very dorsal (DV coordinates of 15 and 16+). These largely nonresponsive sites were tested in every track so as not to "miss" the beginning of the responsive area. Once the electrode entered deeper locations, responses were elicited at a high probability (>80%) of stimulus sites. At the deepest sites, responses were seen at every site tested, and threshold currents were typically quite low.

To estimate the probability, rather than the frequency, of a particular response from midbrain stimulation at each region of the AP, ML, or DV coordinates, the frequency of observation of each response type within each 1- or 2-mm bin was divided by the "total responses" for that bin (Table 2). For example, to calculate the probability of "body plus" responses from stimulation at a region defined by AP 2 to 3, divide 20 (the frequency of occurrences in the "B+" group) by 72 ("total responses" at AP 2, 3 mm). It should be noted that because of the rounding to the nearest integer, the AP 2, 3 mm bin actually extends from 1.50 to 3.49 mm anterior to earbar zero.

The calculation of response probabilities would not have been necessary if there had been a uniform distribution of stimulation sites in the midbrain. The fact was, however, that the central regions of the explored area were sampled more frequently than the periphery. (Note the pattern of numbers in the "total sites" column in the AP and ML dimensions of Table 2). Also, the dorsal regions were sampled more frequently than the ventral regions. Because of this nonuniform sampling, the probability is a better estimate of the likelihood of producing a particular response at a stimulus site than the raw frequency of observation in these data.

RESULTS

Types and Frequency of Responses. Stimulation of the vast majority (92%) of sites could be categorized as resulting in an isolated or combined response of the face, body, jaw, or voice. Responses categorized as "face" included movements of the muzzle, eyelid (raising and lowering), eyeball (rotating, elevating, and depressing), and ear (flattening against the side of the head, pricking up, and rotating). Observations of "jaw" movement involved either raising or lowering of the mandible. "Body" included movements of the neck, shoulder, leg, trunk, and (rarely) tail, either alone or in any combination. "Voice," or vocalization as defined in Methods, included howls, growls, whines, and, rarely, barks. Table 1 enumerates the frequency with which these 4 basic types of responses occurred in isolation ("only") or in their possible combinations. The majority (82%) of stimulus sites resulted in movements of the face or body, either in isolation or in combination with other responses. Voice responses were observed much less frequently. Isolated voice responses (V only group) were observed from 76 stimulus sites. Nine such responses were evoked by currents less than 0.35 mA.

Some stimulus sites (8%) resulted in responses that could not be categorized into 1 of the 4 basic types of responses. These included a variety of respiratory and laryngeal responses: increased depth or rate of respiration, inspiration followed by prolonged expiration, audible expiration (breathy sound produced by turbulent airflow through a partially closed glottis), and adduction, abduction, and lengthening of the vocal folds. Finally, what appeared to be movements associated with "retching" were evoked on several occasions from stimulation delivered close to earbar zero of the stereotaxic coordinate system. This response consisted of alternating opening and closing movements of the glottis and upper esophageal sphincter, accompanied by attempted opening of the mouth. Of interest, this retch-

TABLE 2. FREQUENCY OF OBSERVATION OF RESPONSE TYPES WITHIN BINS OF AP, ML, AND DV HISTOGRAMS

Stimulus Site (mm)	*Total Sites*	*Total Responses*	*B+*	*F+*	*J+*	*V+*	*B(o)*	*F(o)*	*J(o)*	*V(o)*	*V(o) <0.35 mA*
AP											
0, 1	11	7	4	1	5	0	1	0	1	0	1
2, 3	86	72	20	4	21	3	19	1	20	1	2
4, 5	201	164	46	49	59	15	18	22	38	5	1
6, 7	350	256	83	106	71	43	14	39	41	11	5
8, 9	421	266	69	122	54	36	22	58	6	27	0
10, 11	342	214	53	87	40	35	9	59	3	28	0
12, 13	223	134	45	52	26	5	5	61	1	4	0
14, 15	70	42	18	21	10	0	3	18	0	0	0
16, 17	28	3	0	0	0	0	0	3	0	0	0
ML											
1	6	5	0	1	1	0	1	1	0	0	0
2	197	168	60	77	62	8	25	33	16	2	0
3	136	125	39	46	28	5	4	36	15	5	0
4	305	215	78	97	71	18	21	36	26	10	0
5	261	210	59	65	35	22	18	48	21	21	3
6	360	226	58	81	52	42	12	52	15	29	5
7	225	144	28	49	22	37	4	36	7	9	1
8	179	44	15	21	10	5	3	13	3	0	0
9+	68	21	1	5	5	0	3	6	7	0	0
DV											
2	2	2	0	0	0	0	0	0	2	0	0
3	16	16	1	5	6	2	2	3	3	2	0
4	25	25	6	11	6	5	1	6	2	2	0
5	76	73	19	39	27	12	5	12	5	4	0
6	96	87	35	48	31	12	5	13	6	7	3
7	113	104	33	47	34	16	8	24	6	6	4
8	126	116	38	57	38	16	12	26	9	3	1
9	145	130	49	56	39	14	15	28	10	6	0
10	190	167	48	58	43	15	18	39	14	8	0
11	153	128	38	43	25	13	13	21	13	9	0
12	153	114	29	37	18	11	8	22	15	8	0
13	140	87	25	22	11	12	1	26	11	8	1
14	135	62	10	12	5	7	2	20	9	7	0
15	117	37	7	7	3	2	1	13	5	5	0
16+	250	10	0	0	0	0	0	8	0	1	0

Boldface indicates rows in which total number of responses within that range was greater than 5% of total of numbers in Total Responses column. AP — anterior-posterior, ML — medial-lateral, DV — dorsal-ventral, B — body, F — face, J — jaw, V — voice, + — plus (see text), (o) — only (see text).

ing response was evoked at extremely low current levels (as low as 0.02 mA); otherwise, the lowest threshold responses were evoked at 0.05 mA (for voice only and jaw only).

Probabilities of Voice, Face, Body, and Jaw Responses. Frequencies of observations of evoked responses for 2-mm regions for the AP dimension and 1-mm regions for the ML and DV dimensions are listed in Table 2. As explained in Methods, these frequency counts were not considered the best predictors for evoking particular responses at particular stimulus regions. Instead, probabilities of responses were used to evaluate these data. Figures 1-3 illustrate the probability of a particular type of response as a function of the distance anterior (Fig 1), lateral (Fig 2), and dorsal (Fig 3) to earbar zero. The probabilities in Figs 1-3 are included only for regions (indicated by boldface in Table 2) that were tested a reasonable number of times, ie, more than 5% of the total stimuli from all of the bins in that dimension. This restriction was applied because as the number of observations becomes small, the probability becomes "unstable." For example, if there was only 1 observation in a bin, the probability of seeing a particular response would be either 0 or 1.0.

In Figs 1-3, the upper graph illustrates probabili-

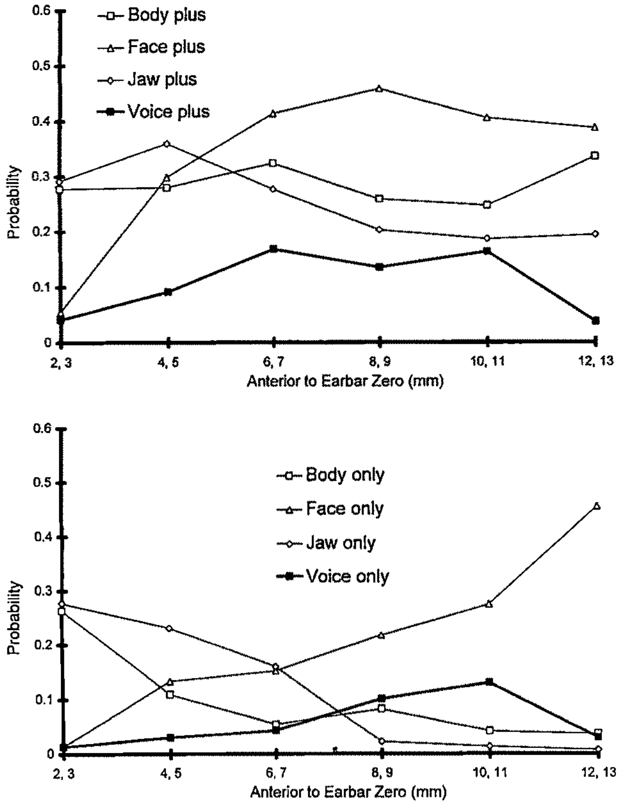

Fig 1. Probability of observing different types of responses as function of distance of stimulation site in anterior-posterior dimension relative to earbar zero. Composition of various types of response groups (body plus, body only, etc) is explained in text. Also see Tables 1 and 2.

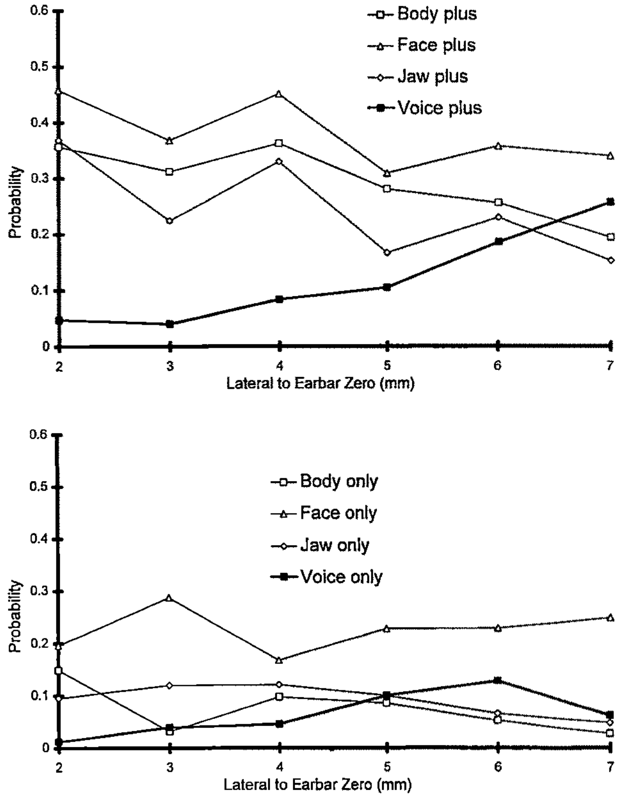

Fig 2. Probability of observing different types of responses as function of distance of stimulation site in medial-lateral dimension relative to earbar zero.

ties for evoking the 4 basic responses in combination with other responses ("plus" groups), and the lower graph shows the probabilities for the 4 basic responses occurring alone ("only" groups). In all dimensions, the "only" response probabilities are lower than the "plus" responses. For the AP and ML dimensions (Figs 1 and 2), the "only" responses appear to be more regionally specified than the "plus" responses. For the DV dimension (Fig 3), the spatial distributions of the "only" and "plus" groups are generally similar.

Along the AP dimension, the pattern of probabilities of evoking responses from stimulation differed markedly for different types of responses (Fig 1). The probability of observing responses of the jaw and body increased with more-posterior stimulus sites, and the probability of observing face responses increased with more-anterior stimulus sites. The probability of evoking voice was greatest between 8 and 11 mm anterior to earbar zero.

From Fig 2, it is evident that the probabilities of evoking body, face, and jaw responses was broadly distributed along the ML dimension. The result was quite different for the voice, however. The probability of observing voice responses was much higher at more lateral stimulus sites (6 to 7 mm lateral to midline). Sites more lateral than 8 mm from the midline were not studied frequently, because our electrode, when aimed at far-lateral tracks, was in danger of being damaged by hitting the skull just lateral to the midbrain. For the DV dimension (Fig 3), the probability of evoking various responses differed little from 5 to 12 mm dorsal to earbar zero.

The probability distributions shown in Figs 1-3 represent responses evoked at a variety of stimulus currents (selected as described in Methods), some of which were fairly high. It is reasonable to ask whether these distributions would change in important ways if these probabilities were calculated only for sites in which responses were evoked by low currents. Figure 4 shows the probability distributions of the "plus" response groups whose stimulus currents were less than 0.35 mA. The configuration of these "low-threshold" distributions is different from the results shown in Figs 1-3 in 2 important respects. First, in the AP dimension (top graph of Fig 4), the probability of evoking face responses was quite low in the most anterior regions, whereas it was quite high in this region when all stimulus currents were considered (top graph of Fig 1). This difference revealed that although "face plus" was the predomi-

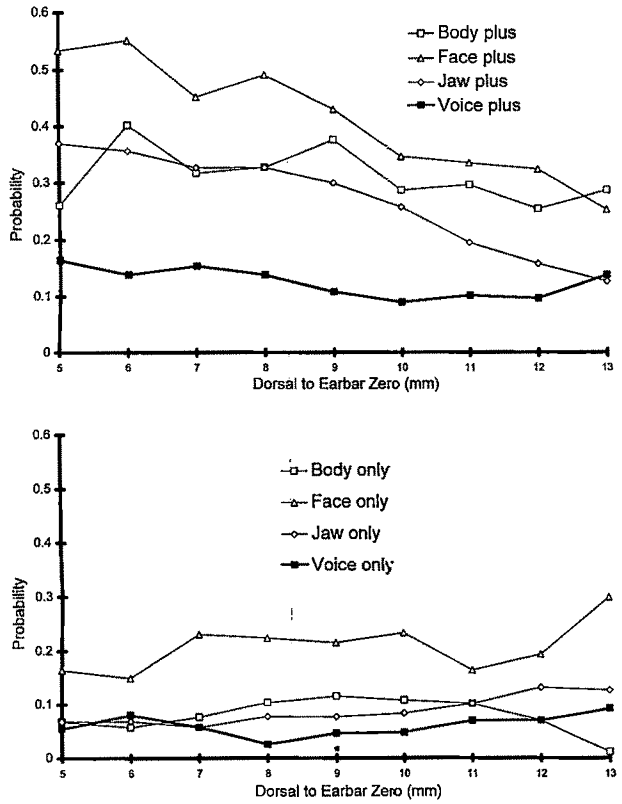

Fig 3. Probability of observing different types of responses as function of distance of stimulation site in dorsal-ventral dimension relative to earbar zero.

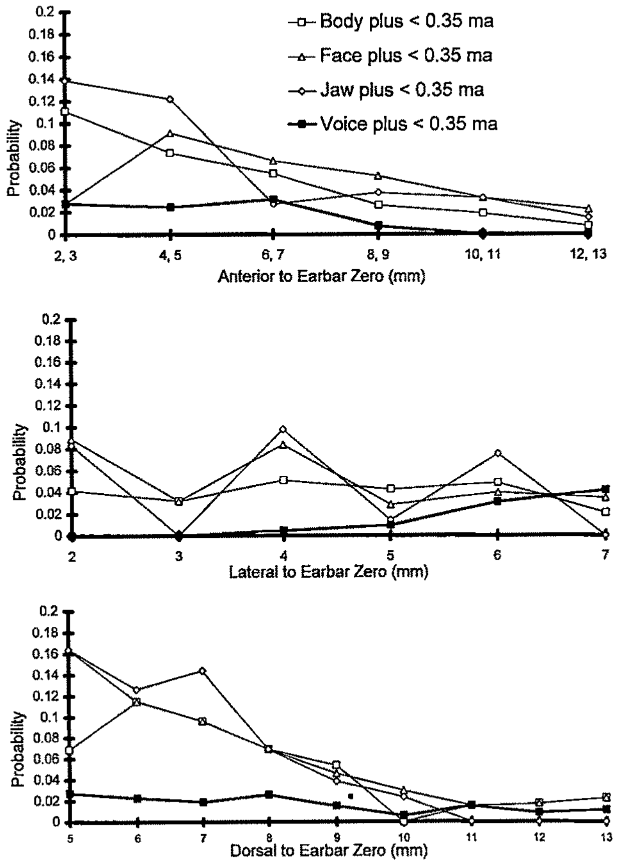

Fig 4. Probability of observing 4 basic types of "plus" responses as function of 3 dimensions relative to earbar zero for low-threshold stimulus sites. Data for graphs are sites at which 1.5-time threshold current level was less than 0.35 mA.

nant response observed in this anterior region, the currents necessary to evoke responses tended to be quite high, ie, 1 to 1.5 mA. The second important difference was that low-threshold responses of all types were primarily confined to depths of 5 to 9 mm dorsal to earbar zero (bottom graph of Fig 4 and top graph of Fig 3). This was particularly true for body plus and jaw plus responses; as noted previously, it was sometimes necessary to limit our stimulus current when stimulating in these "deep" regions to avoid violent movements of the body or jaw.

The data of Fig 4 were based on the "plus" groups because it is desirable to calculate probabilities from a reasonable number of responses. It would obviously be useful, however, to know the location of the stimulus sites that produce low-threshold voice-only responses. There were 9 stimulus sites that produced voice-only responses at currents of less than 0.35 mA. The response probabilities as a function of the coordinates of the stimulus sites are presented in Fig 5. This Figure suggests that the region evoking these "ideal" responses is narrowly distributed in the ML and DV dimensions, but rather broadly distributed in the AP dimension.

DISCUSSION

Vocalization was evoked by electrical stimulation of the midbrain in 8 dogs anesthetized with pentobarbital. Stereotaxic coordinates of the most probable sites for eliciting vocalization were 6 to 10 mm anterior, 6 to 7 mm lateral, and 6 to 8 mm dorsal to earbar zero, although vocalization sites ranged in location from approximately 0 to 17 mm anterior, 1 to 9 mm lateral, and 1 to 16 mm dorsal to earbar zero. In a similarly conducted study by Hsiao et al,[18] stimulation sites for eliciting vocalization in anesthetized dogs were comparable to those reported here, except that their range of observations was limited by the midbrain area explored. Specifically, they explored locations 8 to 12 mm anterior, 3 to 5 mm lateral, and 5 to 20 mm dorsal to earbar zero, and elicited vocalizations at sites 9 to 11 mm anterior, 3 to 5 mm lateral, and 5 to 16 mm dorsal to earbar zero. Unfortunately, Hsiao et al were not able to observe vocalization at the more posterior, ventral, and (especially) lateral sites at which we elicited vocalization.

It is regrettable that the histologic preparation of the brains used in the present study was not adequate for detailed reconstruction of electrode tracks. The

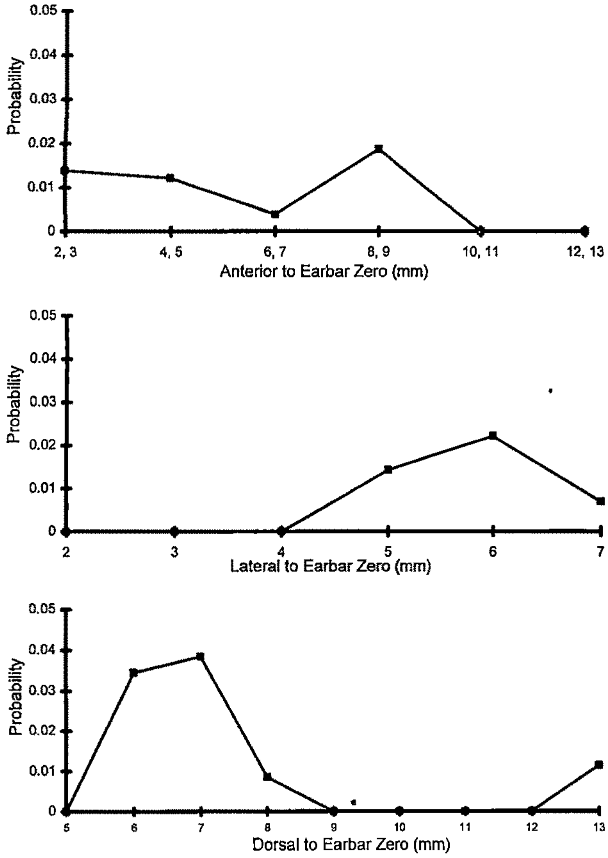

Fig 5. Probability of observing voice-only response at stimulus currents of less than 0.35 mA as function of 3 dimensions relative to earbar zero. There are only 9 stimulus sites (see far-right column of Table 2) used in calculation of these probabilities.

tissue did show, however, that the main concentration of electrode tracks was located in the area predicted by the canine stereotaxic atlas of Dua-Sharma et al.[26] Although the area from which vocalization could be evoked was extensive, it did not seem to include regions close to the midline of the midbrain. According to the stereotaxic atlas, the midbrain PAG extends only about 3 mm lateral to the midline for any point in the midbrain. We seldom observed vocalization from stimulation sites less than 4 mm lateral to midline. It seems unlikely, therefore, that the vocalization we observed resulted from stimulation of somata of neurons in the PAG. Vocalization in this study may have been the result of stimulation of PAG axons coursing ventrally and laterally through the midbrain tegmentum. According to the canine brain atlas, the low-threshold stimulus sites for vocalization (Fig 4), and in particular those that evoked *only* vocalization (Fig 5), were located in a region of the midbrain very close to the medial lemniscus at the level of the superior colliculus. This conclusion is consistent with 2 earlier electrical stimulation studies in the cat. Magoun et al[4] and Kanai and Wang[9] both illustrated stimulus points very close to the medial lemniscus that produced vocalization without other associated movements. Also, Holstege[27] found labeled neurons near the medial lemniscus, as well as in the PAG, after horseradish peroxidase injections in the nucleus retroambiguus (NRA). The NRA is very probably involved in producing vocalization from chemical stimulation of the PAG.[6]

Our primary interpretation of these observations is that the basic anatomic organization of the midbrain response to electrical stimulation in the dog is similar to that in the cat and monkey. The probable neural system involved is the projection of PAG neurons to lower centers, probably the NRA. Under barbiturate anesthesia, however, the PAG neurons themselves seem remarkably resistant to electrical stimulation. Vocalization can be produced over an extensive area of the lateral tegmentum by using relatively high currents. This is probably because the current spreads far enough to excite PAG axons, which are dispersed over a large area. The extensive DV range over which stimulation was able to produce movements and vocalization (Fig 3) may result from the general ventral and lateral course of these axons. These axons may form a much denser collection close to the medial lemniscus, however, thus accounting for the more restricted range of low-threshold stimulation sites. The observations of Holstege[27] (see Figure 1C,D,E in his report) also suggest the possibility of relay neurons in this pathway located just above the medial lemniscus.

Motor responses other than vocalization elicited by electrical stimulation of the midbrain have been reported to include opening and closing of the mouth, elevation of the tongue, dilatation of the nostrils, retraction of an angle of the mouth, wrinkling of the forehead, and retraction and flattening of the ear in cats and monkeys,[4] extension and flexion of legs and movement of the body in monkeys,[1] and respiratory effects in cats and monkeys.[1,4] All of these responses were observed in the present experiment except movement of the tongue. The tongue was fixed by a brace, however, so we cannot exclude the possibility that these stimuli produced activation of tongue muscles. In addition, movements of the eyelid and eyeball were observed.

The patterns of the probabilities of evoking the 4 basic types of movements illustrated in Figs 1-5 suggest some guidelines for choosing electrode track coordinates when searching for low-threshold sites for producing vocalization. Clearly, lateral locations (5 to 7 mm) are better than more-medial locations. There was a significant amount of variability in the AP dimension, probably due to variation in the angulation of the bony auditory meatus in relation to the cranial base. Generally, however, AP 8 would be a good starting location. On the basis of other responses, one could predict whether the location was too far anterior or posterior in a particular dog. If mainly high-threshold face responses and no jaw responses were observed, then one could predict that the track was too far anterior. If, on the other hand, one were to observe jaw responses and body responses but few face responses, then the track would probably be too far posterior.

Very discrete low-threshold jaw-closing responses were produced by tracks that were posterior and medial to the most probable voice region; these probably are from stimulation of axons of cells from the mesencephalic nucleus of the fifth cranial nerve (MesV; see Figure 1 of de Lanerolle and Lang[28] and Figure 4D of Luschei[29]). It is worth noting, however, that powerful jaw closing responses were elicited by extremely lateral electrode locations. These resulted from current spread to the root of the fifth nerve; this was ascertained in 1 dog by inserting needle electrodes in the temporalis muscle and observing that the electromyogram exhibited a synchronized action potential a few milliseconds after each electrical stimulus. If the jaw movements were produced by spread to the axons of the MesV, the jaw muscle electromyogram would be an interference pattern. Finally, a preponderance of low-threshold body movements is probably a warning that the electrode is approaching the base of the midbrain; these responses may very well represent current spread to the pyramidal tract underlying the medial lemniscus.

In conclusion, a region for elicitation of vocalization by electrical stimulation was identified in the canine midbrain. This region appears to be located near the medial lemniscus at the level of the superior colliculus. Within this region, natural-sounding vocalizations without other motor responses were elicited in the anesthetized dog. The vocalization region apparently is composed of axons arising from the midbrain PAG. The functional relationships between stimulation sites resulting in vocalization and those resulting in other motor responses were described. These can serve as clues for locating the midbrain vocalization region efficiently. These results should aid in the implementation of future investigations of laryngeal physiology.

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IMAGING CASE STUDY OF THE MONTH

MAGNETIC RESONANCE FEATURES OF NASOPHARYNGEAL TERATOMA (HAIRY POLYP) IN AN ADULT

Luis Cerezal, MD
Liencres, Spain
Ana Canga, MD
Liencres, Spain

Carmelo Morales, MD
Torrelavega, Spain
José Gómez, MD
Santander, Spain

Faustino Abascal, MD
Santander, Spain
Manuel Bustamante, MD
Santander, Spain

INTRODUCTION

Dermoids or hairy polyps are uncommon lesions affecting the head and neck region. They are composed of tissues derived from ectoderm and mesoderm. Most originate in the oronasopharyngeal region, being in almost all cases diagnosed in the immediate newborn period.[1] We present the magnetic resonance imaging (MRI) findings of a nasopharyngeal dermoid in an adult patient. This is, to the best of our knowledge, the first demonstration of a nasopharyngeal dermoid in an adult by MRI.

CASE REPORT

A previously healthy 50-year-old man presented with a history of recurrent nasal bleeding. Cavum examination revealed a whitish, shiny, pedunculated mass in the left superior aspect of the soft palate. The patient was referred for MRI to characterize the mass. The MRI demonstrated a pedunculated, rounded, and well-defined mass in the inferior left lateral wall of the cavum. The lesion showed high signal on T1-weighted images (Fig 1) and intermediate signal on T2-weighted images (Fig 2), being isointense with fat in all the pulse sequences. The central area of the tumor displayed a linear zone of intermediate signal, isointense with muscle. There was no enhancement with gadolinium (not shown). Additionally, a pituitary macroadenoma with suprasellar and right cavernous sinus extension was incidentally identified. A biopsy of the palatal lesion was performed, with the histologic examination revealing keratinized stratified squamous epithelium and sebaceous glands. Definitive surgical excision through a transoral approach was performed. On gross examination, the mass had an elastic consistency, showing abundant hair follicles on its surface. Microscopically, the tumor was covered with keratinized squamous epithelium with hair follicles and sebaceous and eccrine glands resembling normal skin tissue. The tumoral matrix was composed of mature adipose tissue and striated muscle fascicles. No malignant elements were identified (Fig 3). The pathologic diagnosis was mature teratoma, hairy polyp type.

DISCUSSION

Teratomas are uncommon neoplasms composed of tissue elements foreign to the organ and the anatomic site of origin. They are thought to arise from misplaced pluripotential, primordial germ cells. A teratoma is not necessarily composed of tissues derived from all 3 germ layers; the designation of teratoma can be appropriate even for a lesion with tissues derived from only a single embryonic germ layer, if the tumor shows histologically divergent differentiation.[2]

Teratomas have been classified into 4 types by Arnold[3] and Schwalbe.[4] Dermoids or hairy polyps are composed of epidermoid and mesodermal tissue elements. They consist mainly of fibroadipose tissue, with fragments of striated muscle, cartilage, or bone, and are usually pedunculated and covered with skin and often include hair. Most nasopharyngeal teratomas fall into this category. The second type is teratoids. They contain tissue elements of all 3 germinal layers, but are not well differentiated and lack clear organization. True teratomas are the third type. They also contain ectodermal, mesodermal, and endodermal elements; however, they are larger, have more

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From the Department of Radiology, Santa Cruz Hospital, Liencres (Cerezal, Canga), the Department of Otorhinolaryngology, Sierrallana Hospital, Torrelavega (Morales), and the Departments of Radiology (Abascal, Bustamante) and Pathology (Gómez), University Hospital Marqués de Valdecilla, Santander, Spain.

CORRESPONDENCE — Manuel Bustamante, MD, Servicio de Radiodiagnóstico, Hospital Universitario Marqués de Valdecilla, Av. de Valdecilla s/n, Santander 39008, Spain.

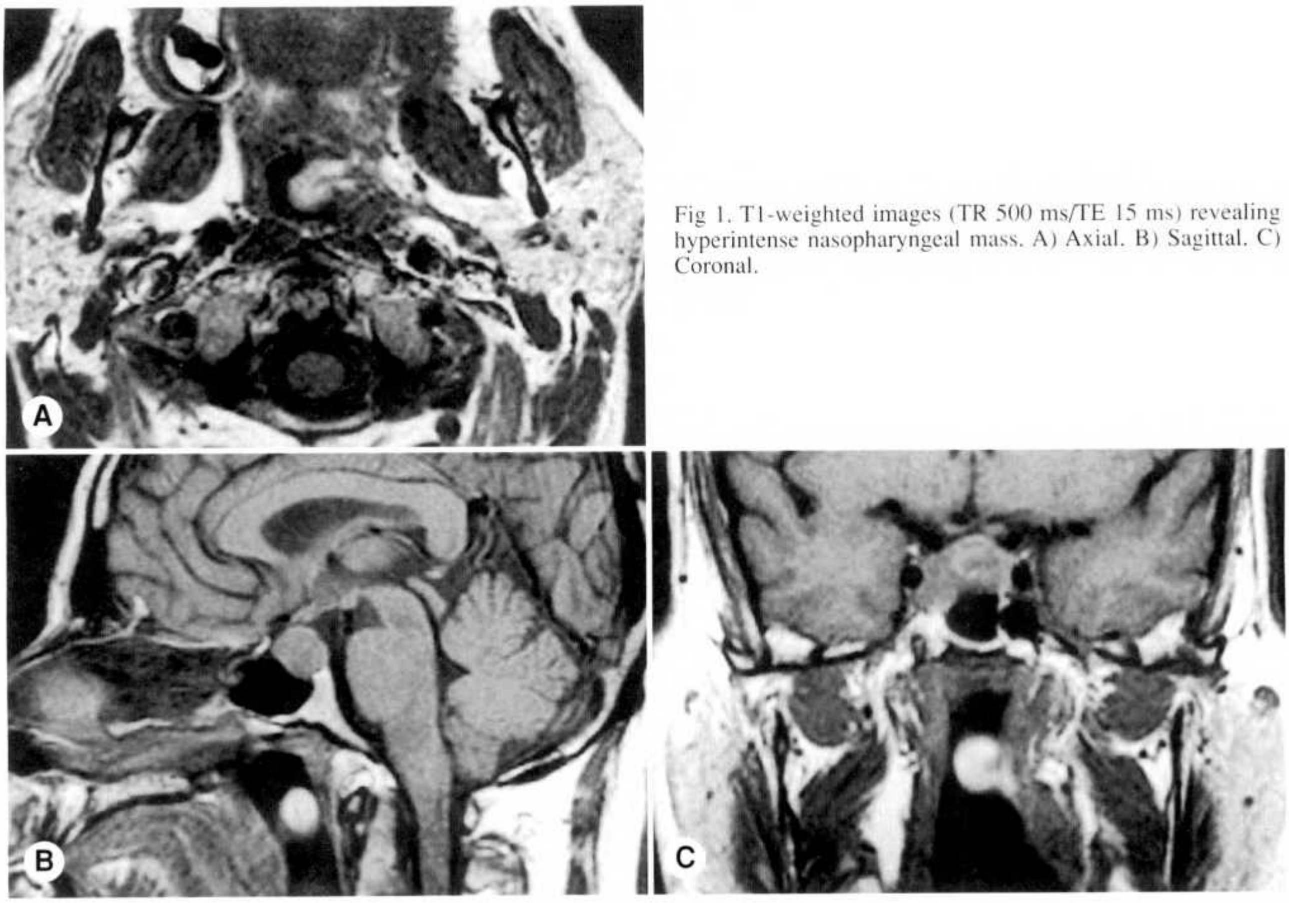

Fig 1. T1-weighted images (TR 500 ms/TE 15 ms) revealing hyperintense nasopharyngeal mass. A) Axial. B) Sagittal. C) Coronal.

organization, and are differentiated to the degree that organs can be recognized by histologic structure. The last type, epignathi, are the least frequent and represent the highest degree of differentiation into a parasitic fetus, with gross development into organs and limbs; they are generally incompatible with life.

The incidence, for all 4 types, is 1 per 4,000 births.[5-8] Teratomas, as a group, are the most common congenital tumors, accounting for up to 36% of all cases.[2] There is a 5 or 6 to 1 female-to-male predominance.[5,9,10] The majority of teratomas occur in the sacrococcygeal area, gonads, retroperitoneum, and anterior mediastinum. Fewer than 5% occur in the head and neck, in which the most common site is the cervical region, followed by oropharyngeal and nasopharyngeal locations.[5,8,10]

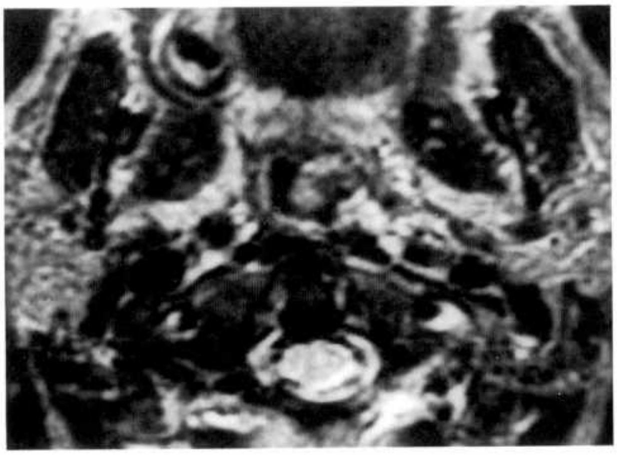

Fig 2. Axial T2-weighted image (TR 2,500 ms/TE 90 ms) revealing nasopharyngeal mass with intermediate signal isointense with fat.

Hairy polyp or dermoid of the nasopharynx was first described by Brown-Kelly.[11] Pharyngeal dermoids are within the spectrum of teratoma. However, more recently, some authors have considered them to be choristomatous accessory auricles.[12,13] The distinction is clinically relevant, since choristomas do not have growth or malignant potential, in contrast to some teratomas. Support for this hypothesis is provided by the presence of cartilage identical to auricular cartilage in some of these lesions, and the location of dermoid polyps. Most are located laterally in the nasopharynx, not far from the eustachian tube, and some even arise within the tube orifice, or in the middle ear space.[12] In our case, the location in the nasopharyngeal surface of the soft palate and the absence of cartilage go against this hypothesis.

The clinical presentation depends on the size and location of the tumor. Most nasopharyngeal terato-

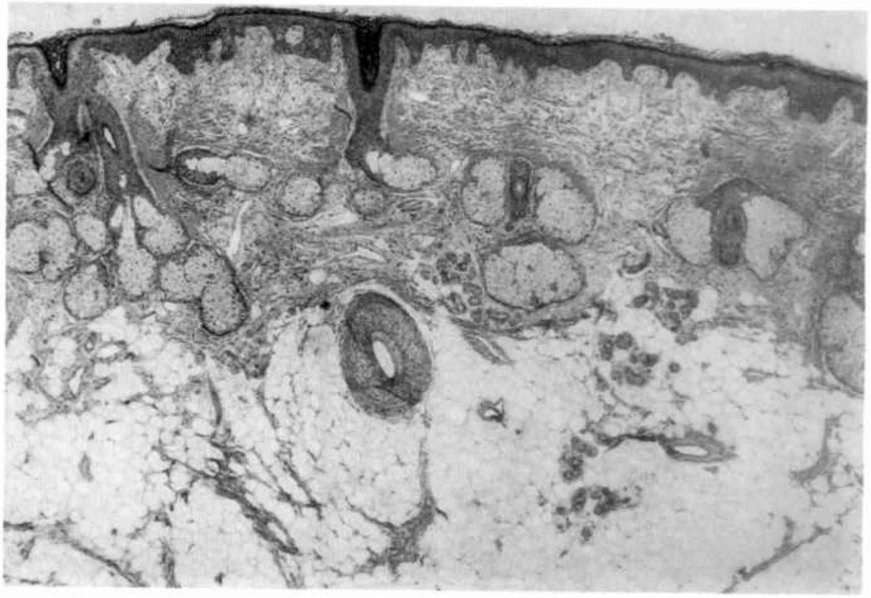

Fig 3. Photomicrograph of tumor revealing stratified squamous epithelium with mature skin appendages. Below, adipose tissue and striated muscle fibers are seen.

mas present during the neonatal period with symptoms of respiratory distress. Dermoids, which are usually pedunculated, may cause only partial airway obstruction, manifested by snoring, dysphagia, and rhinorrhea.[10] In our case, recurrent nasal bleeding was the only clinical manifestation.

Benign and malignant forms of teratoma can exist, although they are not always easy to distinguish. They are also classified as mature or immature. Clinically benign teratomas of the head and neck region may contain immature tissue in both infantile and adult tumors.[7] Several authors have reported that the finding of immature tissue in teratoma is of limited prognostic significance and should not be equated with malignancy.[14,15] Malignant degeneration, which is relatively common at other sites, is extremely unusual in the nasopharyngeal region. No report has been published in the English-language literature of malignant nasopharyngeal teratoma in childhood. Pospiech et al[16] reported a case of malignant nasopharyngeal teratoma in an adult.

The differential diagnosis of nasopharyngeal masses in the adult includes chordoma, craniopharyngioma, nasal polyp, juvenile angiofibroma, Thornwaldt's cyst, hamartoma, hemangioma, lipoma, carcinoma, and lymphoma. Location, MRI signal behavior, and the absence of gadolinium enhancement allowed us to exclude most of these lesions, leaving teratoma and lipoma the most likely diagnoses.

Computed tomography and MRI are helpful in delineating the extent of the mass, including the intracranial extension. Sagittal and coronal images of MRI are especially useful in evaluating the separation of the central nervous system from the nasopharyngeal mass. Also, MRI can depict associated central nervous system anomalies.[6,10] In the case we present, the finding of a pituitary macroadenoma was purely casual. Teratomas are generally heterogeneous lesions, with calcifications, fat, and soft tissue areas. With computed tomography, a teratoma can be suspected when finding a mass with calcifications and areas of low attenuation representing lipid. On MRI, they usually appear as high-intensity masses on T1-weighted images, due to their high fat content, with punctate foci of low signal corresponding to calcium. Contrast enhancement is not usually seen, unless areas of malignant degeneration are present. The MRI findings in our case correlated with the histopathology, showing high signal on T1 weighting due to high fat content of the tumoral matrix and a linear, intermediate-signal central zone representing radially striated muscle fibers. No calcifications were found.

The definitive treatment is complete surgical resection. Pedunculated masses, as in our case, can be easily snared, but sessile forms may be difficult to excise because of limited accessibility, so that in some cases more aggressive surgical approaches are necessary.[5] These tumors rarely recur; if persistent, they may be resected a second time.[6]

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CLINICOPATHOLOGICAL CONSULTATION

PREGNANCY AND MALIGNANT NEOPLASMS OF THE HEAD AND NECK

ALFIO FERLITO, MD
UDINE, ITALY

STEPHANIE L. DEVANEY, RN, BSN
ANN ARBOR, MICHIGAN

ANTONINO CARBONE, MD
AVIANO, ITALY

MICHELE MAIO, MD
AVIANO, ITALY

KENNETH O. DEVANEY, MD
CHICAGO, ILLINOIS

ALESSANDRA RINALDO, MD
PADUA, ITALY

IMRICH FRIEDMANN, MD, FRCPATH
HARROW, ENGLAND

This paper, based on a literature review, deals with the occasional development of a head and neck neoplasm in the pregnant woman. This rare event makes for some challenging problems in patient management, insofar as the otolaryngologist is actually responsible for taking care of 2 patients — the mother and her unborn child. In particular, 4 tumor types have figured prominently among those head and neck tumors arising in pregnant women: cancer of the larynx, cancer of the thyroid, malignant melanomas, and malignant lymphomas of the head and neck. Of these, the most common appear to be melanomas, followed by lymphomas, thyroid carcinomas, and, finally, laryngeal carcinomas. The thyroid tumors, lymphomas, and laryngeal carcinomas do not appear to behave more aggressively in pregnant than in nonpregnant patients; there is, however, some anecdotal evidence to suggest that melanomas in pregnant women may be more aggressive neoplasms than similar-stage tumors in nonpregnant women. One difficulty in treating any of these tumor types in this clinical setting is the limitation that may be imposed on the use of adjuvant therapy by the presence of the unborn child, which may put the attending physicians in the difficult position of balancing less aggressive therapy for the mother against the potential for harming the baby through use of toxic systemic therapy.

INTRODUCTION

The management of pregnancy accompanied by malignant neoplastic disease may present considerable problems, not only of a diagnostic and therapeutic nature. There are also moral, ethical, and even religious issues to be considered.

Malignant neoplasms may be present and be diagnosed at pregnancy or may have preexisted and been reactivated by pregnancy. These and some related aspects will be discussed on selected examples of malignant neoplasms of the head and neck, such as cancers of the larynx, cancers of the thyroid gland, malignant melanomas, and malignant lymphomas of the head and neck. Brief reference will be made to some histopathologic features, and the therapeutic details will be discussed as applied to the neoplasms included in this consultation report.

The adequate treatment of an individual case requires the close collaboration of various experts, and the unusual occurrence and stealthy onset of these neoplasms may often delay the diagnosis. The surgical treatment may be based on some general rules, but each case must be considered on its own merits, and the potential benefits of immediate treatment should be weighed against the damage to the fetus that might ensue. This applies also to general anesthesia, to be discussed.

PREGNANCY AND CANCER OF LARYNX

Laryngeal cancer is a relatively uncommon disease, but it is the most frequent head and neck cancer, apart from skin malignancies. It occurs most frequently in men during the sixth and seventh decades of life, though recent findings show that more than 20% of cases now occur in women.[1,2] The increase in the incidence of laryngeal cancer among women has been proportionally greater than among men,[3] probably due to changes in smoking and drinking habits among women in recent years. Nonetheless, the age and sex of the pregnant woman makes her an unlikely candidate for laryngeal malignancies, and only 10 cases of laryngeal cancer in pregnancy have been reported in the literature.[4-13]

From the Department of Otolaryngology–Head and Neck Surgery, University of Udine, Udine (Ferlito), the Division of Pathology (Carbone) and the Advanced Immunotherapy Unit (Maio), National Cancer Institute, IRCCS, Aviano, and the Department of Otolaryngology, University of Padua, Padua (Rinaldo), Italy, the Department of Pathology, University of Michigan, Ann Arbor, Michigan (S. L. Devaney), the Department of Pathology, University of Chicago, Chicago, Illinois (K. O. Devaney), and Northwick Park Hospital and St Mark's Hospital, Harrow, England (Friedmann).

CORRESPONDENCE — Alfio Ferlito, MD, Dept of Otolaryngology–Head and Neck Surgery, University of Udine, Policlinico Città di Udine, Viale Venezia 410, 33100 Udine, Italy.

The ages of these patients ranged from 20 to 40 years (mean 28.4 years; median 27.5 years), and hoarseness was their main symptom. Four patients denied smoking, while 3 were heavy smokers. The gestation time at presentation ranged from 4 weeks to 7 months. In 1 case, the diagnosis of laryngeal squamous cell carcinoma preceded that of pregnancy,[7] and in another case it followed delivery.[10] All the malignant neoplasms were squamous cell carcinomas, with the exception of 1 case of epithelial-myoepithelial carcinoma.[9] The glottic region was the most often involved, and all but 1 of the patients had negative lymph nodes. (One presented local and distant metastases to the bones.[11]) Surgical treatment and radiotherapy were applied in different combinations during the pregnancy and/or after the delivery. Chemotherapy was used in 1 patient after the delivery.[11] Nine women were reported to be alive and well with no evidence of disease at their last follow-up. Only 1 patient died, of carotid hemorrhage 2 years after treatment.[11]

Three patients underwent a secondary tracheoesophageal puncture and the insertion of a voice prosthesis, producing an intelligible voice.[14]

The coexistence of paraganglioma and pregnancy has also been reported in the larynx,[15] as well as in extralaryngeal areas,[16,17] but this lesion is considered benign when it occurs in the larynx.[18]

The endocrine changes occurring during pregnancy have a wide range of well-documented effects all over the body, especially in the abdomen, but any laryngeal involvement has yet to be clearly established. Pregnancy may be accompanied by hoarseness, vascularization, and enlargement of the true and false vocal cords, occasionally associated with an altered mucus secretion, leading to laryngitis sicca gravidarum. After delivery, these symptoms disappear.[19]

The treatment of laryngeal cancer is still controversial, and pregnancy makes therapeutic decisions even more difficult. The risk of teratogenesis is higher during the first trimester of pregnancy (ie, during organogenesis), but the teratogenic risks of anesthesia are negligible by comparison with those of irradiation or chemotherapy. Typical anesthetic agents readily reach the fetus, but are not known to be teratogenic in humans. Alternatively, radiotherapy may be administered (while shielding the patient's abdomen and the fetus from the radiation). Radiotherapy should not be used to treat laryngeal cancer in patients under 40 years of age, since such treatment could increase the likelihood of a second primary laryngeal cancer at the site of irradiation or in neighboring structures.

There is no significant difference in actuarial survival between pregnant and nonpregnant patients with laryngeal tumors of equivalent stage and identical phenotype. Pregnancy and the biologic influence of female hormones do not appear to affect the natural course of laryngeal cancer.

PREGNANCY AND CANCER OF THYROID

The observation that thyroid neoplasms, as a group, are more frequently encountered in women than in men may suggest a potential link between the hormone status of female patients and the onset of these tumors. Moreover, there appears to be a genuine increase in the frequency of diagnosis of thyroid neoplasms during pregnancy, though it is difficult to assess whether this represents a real increase of incidence or merely an increased likelihood of their being recognized. The management problems presented by thyroid malignancies in pregnancy are genuine, nonetheless.

It has been estimated that approximately 1 in 1,000 pregnancies is complicated by the development of a malignant tumor in the mother; only a small proportion of this small number is represented by primary thyroid malignancies.[20] According to a survey by the New Mexico State Cancer Registry, there were in a 20-year period 22 cases of thyroid cancer in pregnant women and 464 in nonpregnant women 18 to 46 years of age, that is, approximately 1 new case of thyroid cancer in pregnant women annually.[21]

Pregnant women with malignant tumors of the thyroid can be divided into 2 categories: women with thyroid tumors who become pregnant and women whose thyroid masses are discovered during pregnancy. Women who are evaluated for solitary thyroid nodules while pregnant (most often in their third trimesters) apparently form a distinct patient population: in some observers' experience, approximately 40% of such patients prove to have benign thyroid adenomas, while in a similar proportion (40%), thyroid malignancies are ultimately diagnosed.[22-25]

A clinical note worth mentioning relates to the possibility of inherited thyroid disease, or the multiple endocrine neoplasia (MEN) syndromes. While medullary carcinomas are decidedly uncommon in pregnant women, the diagnosis of such a lesion should prompt evaluation of the patient and her family for evidence of MEN 2a (Sipple's) syndrome or MEN 2b (MEN 3) syndrome.[26,27]

The full range of thyroid malignancies covers a variety of tumor types, from low- to high-grade lesions. In practical terms, however, the vast majority of thyroid malignancies diagnosed in pregnant women have been papillary carcinomas, which have been seen 10 times more frequently than the next most frequent histologic pattern, ie, follicular carcinoma. The remaining histologic types — medullary carcinomas, anaplastic carcinomas, malignant lymphomas, sarcomas, and metastases — are rare enough to be considered curiosities in pregnant women and are therefore not further explored as pathologic entities here.[28]

Although not common in pregnancy, solitary nodules first noted in pregnant women are about 3 times more likely to be malignant than solitary nodules in nonpregnant women of similar age.[29-36] Women whose thyroid cancer has been diagnosed before pregnancy exhibit no modification in the course of their disease as a result of pregnancy.[37,38]

To date, neither estrogen nor progesterone receptors have been identified in cancers of the thyroid.[39]

Evaluating a thyroid tumor in a pregnant woman differs from that in a nonpregnant patient. For example, the diagnostic (and, for that matter, therapeutic) use of radioactive iodine compounds is discouraged in pregnant patients in view of the very real risks to the fetus. The mainstays for the evaluation of these patients are ultrasonographic study of the mass (to distinguish cystic from solid lesions), fine needle aspiration biopsy and cytologic study of the aspirated material, and thyroid function studies.[22,40]

The histologic subtypes of thyroid neoplasms are important to the clinician coping with the simultaneous management of 2 conditions (the neoplasm and the pregnancy). Papillary carcinomas are generally viewed as tumors with a propensity for metastasizing via the lymph nodes, while follicular carcinomas preferentially metastasize by hematogenous pathways.

Once the diagnosis of thyroid malignancy has been established during pregnancy, surgery should be performed.[32] The pregnancy does not appear to affect the growth of thyroid cancer.[25]

PREGNANCY AND MELANOMA

The incidence of cutaneous melanoma has been increasing worldwide,[41] and melanoma develops in more than one third of women of child-bearing age.[42] These figures, together with previous case reports suggesting that pregnancy may have an adverse effect on the clinical course of melanoma, have recently stimulated several controlled studies focusing on the potential relationship between pregnancy and the pathogenesis, incidence, and prognosis of malignant melanoma. Malignant melanoma complicating pregnancy has an estimated incidence ranging from 0.14 to 2.8 cases per 1,000 deliveries,[43] and accounts for 8% of all malignancies diagnosed during pregnancy.[44] However, with the trend toward pregnancies at an older age and the steady increase in the incidence of melanoma, these figures are likely to be higher and increasing in the future.

Changes in melanocytic nevi are believed to be associated with pregnancy,[45] and pregnant women have reported subjective observations of preexisting melanocytic nevi becoming darker and larger.[46] These observations and the skin hyperpigmentation associated with the use of oral contraceptives[47] have supported the belief that the clinical course of malignant melanoma may be adversely affected during pregnancy. Its regression following delivery or abortion,[48-50] and occasional increase in the thickness of melanomas arising during pregnancy,[51] suggest a potential influence of female estrogens and progesterone on the growth rate of melanocytes and melanoma cells. Objective alterations of the diameter have only been reported in 8 of 129 (6.2%) nevi examined in 22 pregnant women.[52] No correlation has been found between oral contraception and the risk of melanoma development.[53] Last, immunohistochemical analysis failed to demonstrate estrogen and progesterone receptors in 14 cases of pregnancy-associated primary or metastatic melanomas,[54] suggesting that estrogen-binding proteins, previously detected on melanoma cells by biochemical techniques, do not represent well-defined estrogen receptors. The lack of any specific binding and subsequent biologic effects of estrogens on melanoma cells is also supported by the recent demonstration that the antiproliferative activity of tamoxifen is not mediated by its anti-estrogenic properties, and that tamoxifen binds to type II estrogen receptors[55] that show low affinity for estrogens. Consistent with these observations, tamoxifen greatly enhances the efficacy of antineoplastic regimens currently adopted for the treatment of metastatic melanoma.[56]

In spite of numerous case reports and uncontrolled studies suggesting that pregnancy may induce melanoma and/or worsen its prognosis, recent controlled studies have shown no statistically significant difference in the survival of patients developing melanoma in the course of pregnancy and appropriate controls[57]; when the disease-free interval (DFI) was analyzed, it was found to be significantly shorter for pregnant patients in 2 of 4 studies of DFI.[57] The shorter DFI was attributed to a shortened time to nodal metastasis, and elective lymph node dissection was suggested. However, a more conservative approach may be advisable. One study has shown no differences in histologic type or anatomic site of primary melanomas developing in pregnant women compared with nonpregnant controls.[51] That the mean thickness of pregnancy-associated melanomas was significantly greater than that of tumors unassociated with pregnancy suggests the potential influence of hormonal factors on the phenomenon.

Historical reports of "recurrent fulminating melanomatosis" occurring in pregnant women shortly after treatment for melanoma,[58] together with anecdotal reports of melanomas with a worsening clinical course during pregnancy and with clinical regression of disease between pregnancies,[59] led investigators to recommend surgical sterilization to prevent additional pregnancies. However, 2 more recent studies indicated no difference in survival rates or DFI between women who became pregnant after treatment for primary melanoma and control groups.[42,60]

A better 5-year survival rate for women with prior pregnancies than for those without led to the hypothesis that pregnancy may generate a protective immune response against melanoma antigens cross-reactive to fetal antigens.[61] However, further and more extensive controlled studies found either no influence of previous pregnancies on survival and DFI in women diagnosed with primary melanoma,[60,62] or a better survival only for women who had 5 or more pregnancies before a primary melanoma was diagnosed.[63] Though limited in number, these studies strongly suggest that prior pregnancies do not have an adverse effect on the clinical course of the disease, once primary melanoma is diagnosed.

When malignant melanoma develops in a pregnant woman, adequate surgical management is similar to that of melanomas unassociated with pregnancy, and depends on the thickness of the tumor. Due to its limited efficacy in delaying recurrence,[64] elective regional lymph node dissection is not advisable in patients with melanoma of the lower limbs. Sentinel lymph node biopsy should be performed, and if it proves positive for micrometastases, regional lymph node dissection must not be delayed until delivery. Morton et al[65] found that the sentinel node could be identified in 80% of patients by injecting a vital blue dye intradermally around the primary tumor. A negative sentinel lymph node on biopsy is an acceptable indicator that the nodal basin has not been affected by the neoplasm.[66] Sentinel node biopsies have several advantages, and a negative result will reduce the extent, cost, and morbidity of surgery for many melanoma patients who would otherwise be subjected to elective lymph node dissection.[66]

Additional diagnostic procedures should be limited to serum liver function tests, chest radiography, and accurate physical examination. Regional lymph node metastases developing during pregnancy in patients who had a primary melanoma removed before becoming pregnant should also be surgically excised before delivery.

Immediate therapeutic intervention for systemic disease developing during pregnancy is debatable, due to the limited efficacy of the therapeutic procedures available on the clinical course of the disease and to their known or potential adverse effects on the fetus. The interruption or continuation of the pregnancy should be discussed in the light of the realistic possibilities of achieving disease regression by treatment and in light of the extremely rare possibility of melanoma transmission to the fetus across the placenta, which can be associated with spontaneous tumor regressions in infants born with transplacental metastases.[67]

Women who want to become pregnant after the diagnosis and adequate surgical excision of a primary melanoma should be advised to delay pregnancy for 2 to 3 years (according to tumor thickness), ie, the interval during which their disease is at greatest risk of relapse. Women who have undergone locoregional lymph node dissection should be advised to delay pregnancy for at least 5 years, due to the very high risk (>50%) of developing systemic disease during this period of time. For women with surgically excised metastatic disease, giving medical advice is significantly more complex, in view of the very high risk of early recurrence and the poor 5-year survival rate at this stage of disease; these women should be advised to delay pregnancy for at least 5 years. Birth control methods should be suggested in all these clinical settings, including oral contraception, since this does not seem to affect melanoma development or progression.[53]

On the strength of the epidemiologic and biologic data available, it can be reliably suggested that pregnancy neither influences the onset of primary melanoma nor exacerbates the clinical course of melanoma arising during pregnancy.[68] A positive history of diagnosis and treatment for primary and/or metastatic melanoma should not necessarily prevent a woman from becoming pregnant, provided that a sufficient period of time has elapsed to reduce the risk of recurrence; abortion is not medically indicated, should pregnancy occur during this period.

PREGNANCY AND LYMPHOMAS

The overall incidence of lymphomas complicating pregnancy has not been accurately established.[69,70] Hodgkin's disease (HD) has been reported in 1 in 1,000 to 1 in 6,000 deliveries; this incidence makes it the fourth most common cancer diagnosed during pregnancy.[70,71] In contrast, the association of non-Hodgkin's lymphoma (NHL) with pregnancy is rare, with less than 100 cases reported in the literature.[70,72] In a comprehensive review of the literature,[70] 75 cases of NHL associated with pregnancy between 1937 and 1985 were identified. A recent report from a single institution[72] on a further 12 NHL cases observed since 1987 represents the largest series of NHL associated with pregnancy.[72]

In spite of the confusing and outdated nomenclature used in older reports, the histologic types of NHL associated with pregnancy appear to be uniform[73] and display histologic features consistent with high- or intermediate-grade lymphomas,[72-75] with a tendency to involve the organs most stimulated in pregnancy (breast, ovary, and uterus).[73]

Patients with HD diagnosed during pregnancy tend to show favorable HD subtypes, nodular sclerosis being the most frequent histologic subtype.[72]

Because of the limited data available, the natural history of HD and NHL during pregnancy is not well understood.[73] In particular, whether pregnancy affects the course of neoplastic disease has yet to be established. Pregnancy does not seem to affect adversely the course of HD, so treatment of selected HD patients can be relatively safely deferred until after delivery.[71,72] By contrast, some reports have indicated that NHL in pregnancy is most often associated with disseminated disease, aggressive tumor features, and a worse prognosis than in HD.[72,73]

There is an interesting association of rapidly progressing disease in pregnant patients with Burkitt's lymphoma. These findings have led to speculation that there is either a hormonal influence on this form of cancer in pregnant patients or a connection between the physiological state of immunosuppression during gestation and the aggressive features of these tumors.[69] However, the effect of pregnancy on NHL and on lymphoid neoplasms in general is unpredictable, not only because of a number of competing host factors, but also because of the neoplasm's derivation from the host's immune system.[76]

Several studies have suggested that HD alone has little or no effect on the course of gestation and delivery.[70,71] It has been noted that the incidence of first-trimester loss or premature delivery in women with HD was identical to that of pregnant women in a control group.[70,71] Spontaneous abortion and stillbirth rates appear to be no higher than those observed in a normal population. Although it is exceedingly rare,[70] HD metastasizing to the placenta or fetus has been documented 4 times.[71]

Experience in the management of pregnancy complicated by lymphoma is limited, and whether either condition affects the course of the other is still debated. The pregnant woman with a hematologic neoplasm presents several diagnostic and therapeutic challenges for the clinician. Adequate staging and therapy, in particular, can be difficult. The rare occurrence and stealthy onset of these malignancies also often delay their diagnosis, a fact that may adversely affect prognosis.[71] Radiotherapy and the chemotherapeutic agents usually required to treat hematologic malignancies such as lymphomas may have teratogenic potential,[77] although human studies have not confirmed this risk. Fetal morbidity may also be increased in these patients as a result of direct metastatic extension of the disease to the placenta and/or fetus,[78] and hematologic malignancies display a high tendency to metastasize to the placenta. The teratogenic potential of some diagnostic methods and the possible benefit of combined chemotherapy regimens dictate a limited staging workup during pregnancy.

While tomographic scans and isotope studies are contraindicated, abdominal ultrasound is relatively safe and is useful in pregnancy. On this basis, staging should include history, physical examination, routine blood tests, bone marrow biopsies, chest radiography with abdominal shielding, and abdominal ultrasound. The role and safety of magnetic resonance imaging in the evaluation of a pregnant woman with malignant lymphoma have remained doubtful. Staging laparotomy is usually not necessary in these patients.[70,71]

ANESTHESIA AND SURGICAL TREATMENT IN PREGNANCY

Concerning surgical treatment during pregnancy, some rules of management certainly exist, but generalizations may be dangerous and should be avoided. The clinician must weigh the benefits of immediate treatment while minimizing potential damage to the fetus. In addition to the strictly medical facts, moral, ethical, and religious issues should be carefully considered, and the emotional impact of the situation must not be underestimated.

General anesthesia during pregnancy may be influenced by various complications (increased blood volume, increased heart rate and cardiac output, supine positional hypotension, decreased pulmonary function residual capacity, elevated diaphragm, etc). Surgery may not be absolutely contraindicated in the first trimester of pregnancy, provided specific precautions are taken for general anesthesia (paying attention to maternal apnea periods, monitoring the mother's hemodynamic parameters, avoiding any inhalation of stomach contents, and monitoring fetal heart rate after the 16th week of gestation) and in the postoperative period (monitoring uterine activity).

Concerning the anesthetic to be administered in the first trimester, a classic neuroleptanesthesia is always valid. Thiopental sodium is administered as a short-term hypnotic drug, followed by nitrogen protoxide to maintain hypnosis. Fentanyl citrate can be used as an analgesic, in association with haloperidol decanoate for sedation. Finally, succinylcholine chloride is used initially as a muscle relaxing agent to facilitate the intubation procedure, while curariform medication such as pancuronium bromide is used throughout the operation. None of these drugs have revealed any teratogenic effects,[79-82] except for nitrogen protoxide's showing some teratogenic effect in animal models, albeit at very high doses and after a period of prolonged exposure (24 to 48 hours). Minimal doses of an inhalation anesthetic and shorter exposure periods will reduce the danger.

Close cooperation between the otolaryngology specialist, radiotherapist, obstetrician, and anesthesiologist is essential to select the most valid treatment.

CONCLUSION

The adequate treatment of an individual case requires the close collaboration of various experts. The unusual occurrence and stealthy onset of these neoplasms may often delay the diagnosis. The surgical treatment may be based on some general rules, but each case must be considered on its own merits, and the potential benefits of immediate treatment should be weighed against the damage to the fetus that might ensue.

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LETTERS TO THE EDITOR

To the Editor:

I have read the paper by Goldsher et al (*Ann Otol Rhinol Laryngol* 1996;105:868-70) and would like to thank you for publishing such a clinical trial in your journal.

The authors mention in the second paragraph that Jebeles et al of Birmingham published a 14-subject study of preincisional infiltration of tonsils with bupivacaine.[1] They reported another prospective double-blind study published 2 years later on 22 children undergoing tonsillectomy and adenoidectomy.[2] They concluded that local nerve blockade by bupivacaine reduced short- and long-term pain in children undergoing tonsillectomy and adenoidectomy. They also added that additional studies with larger groups of patients would be necessary to enhance their conclusions. I would like to recommend their article[2] to your readers.

I presented a similar clinical trial in 86 subjects, entitled "The Effect of Bupivacaine and Tenoxicam [a product undergoing clinical trials in the United States — Editor] in the Management of Post Tonsillectomy Pain," at the 23rd National Otorhinolaryngology–Head and Neck Surgery Meeting, in Antalya, Turkey, in 1995.[3,4] In that study, I reached the conclusion that the bupivacaine group showed a frank decrease in post-tonsillectomy pain, whereas the tenoxicam group showed pain relief only in the early post-tonsillectomy period.

Mehmet Ziya Özüer, MD
Çamlık mahallesi
2595 sokak Onevler apt 15/8
Denizli 20020
Turkey

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To the Editor:

We found the article by Devaney et al (*Ann Otol Rhinol Laryngol* 1998;107:439-45) on Wegener's granulomatosis of the head and neck very interesting. It gives a comprehensive and up-to-date overview on this puzzling entity. However, in the paragraph dealing with differential diagnosis, we missed one syndrome that is important to keep in mind: nasal cocaine abuse causing an aggressive midline intranasal and pharyngeal destructive process mimicking Wegener's granulomatosis. This syndrome complex was described for the first time in the late 1980s.[1,2] About 12 cases have been published since, all occurring in the United States.[3-6] In all these cases, massive necrosis of midline structures in the upper respiratory tract was observed. The association with heavy nasal cocaine abuse was often diagnosed with significant delay, since drug abuse is frequently denied by the patient. The pathogenesis of midline upper respiratory tract necrosis of this nature may be complex. Ischemic necrosis almost certainly plays a key role due to sympathetic mediated vasoconstriction. Another factor might be phlogistic additives, frequently found in uncleaned cocaine for illicit use.

Recently, we reported the first such case in Europe.[7] In our patient, necrosis was progressive over weeks, and cocaine abuse was denied. Several biopsies showed necrosis and inflammation, but no sign of vasculitis. The differential diagnosis was complicated by moderately elevated serum levels of coarsely granular antinuclear cytoplasmic antibody, making Wegener's granulomatosis the most likely diagnosis. Following rehydration, antibiotics, and nasal debridement, the patient's general condition gradually improved. He left the hospital and refused further treatment. One year later, he presented again. Now there was a palatal destruction of about 1.5 cm in diameter, complete collapse of the nasal dorsum, and almost complete absence of the nasal septum, inferior and middle turbinates, lower ethmoidal cells, uvula, and the major portion of the soft palate. There were no signs of ongoing ulceration or inflammation. Repeated chest radiography findings, renal function, sedimentation rate, and results of antinuclear cytoplasmic antibody testing were normal. Heavy cocaine abuse in the time preceding the first symptoms was now admitted, but had been stopped since that episode.

Successful management of diseases presenting with massive midline necrosis in the upper respiratory tract is dependent on rapid recognition of the specific cause. Any sinonasal or upper respiratory tract ulcerative condition that persists or remains refractory to treatment may be the first manifestation of a potentially lethal drug addiction. In light of our own experience and the reports in the literature, we strongly recommend including nasal cocaine insufflation in the differential diagnosis of patients suspected of suffering from limited Wegener's granulomatosis in the head and neck region. With this syndrome in mind, nasal drug abuse can be confirmed by urine drug screening.

Christian Sittel, MD
Hans E. Eckel, MD
University of Cologne
50924 Cologne
Germany

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To the Editor:

I write in regard to the article entitled "Transection and Occlusion of Lateral Semicircular Canal in Guinea Pigs" by Nam et al (*Ann Otol Rhinol Laryngol* 1997;106:1082-6).

I note several problems with the auditory brain stem response (ABR) stimuli and calibration that may have rendered the study insensitive to auditory changes and possibly uninterpretable. As do most rodents except for chinchillas, guinea pigs have a much higher frequency auditory range than humans and have lower ABR thresholds. Because most sensorineural hearing losses are greatest at the high frequencies, it is critical to appropriately measure these thresholds.

The click stimuli generated by most clinical systems are 100 microseconds in duration; hence, they have a "null point" or no energy at 10 kHz. Further, the earphones of these systems generally "roll off" between 4 and 5 kHz, further restricting, if not eliminating, the energy above that point. For rodent studies, ABRs should generally minimally include some high-frequency tone bursts at 8 kHz, and preferably to at least 14 to 16 kHz. If click stimuli alone are used, they should be no longer than 50 microseconds in duration, so that they have no null point until 20 kHz. Both tone bursts and clicks must be transduced through an earphone that is flat until that point. These earphones must be specially purchased and impedance-matched to the ABR system to avoid nonlinearities. Even if these precautions are followed for click stimuli, the investigator must be aware that if hearing changes in the high-frequency region, the low-frequency region may still yield an ABR response, because clicks provide broadband energy. However, if human parameters and earphones are employed, the animals' high-frequency regions will not be tested at all. This would be the equivalent of testing for human auditory changes but restricting the study to 500 Hz and below.

It is also inappropriate to use human behavioral calibration for intensity reference. Human behavioral thresholds for ABR stimuli are much higher than guinea pig ABR thresholds. The 0-dB normal hearing level reference used for most human clinical equipment and as described in this study is equal to 30 to 35 dB peak sound pressure level, depending on laboratory norms. However, guinea pigs and rats can provide ABR responses down to 0 dB peak sound pressure level. Therefore, the authors would have missed the first 30 to 35 dB of change, in addition to missing the high-frequency information. Consequently, it is not surprising that the authors detected no auditory changes except for 1 case of profound deafness. Their measurement techniques were not appropriate and therefore cannot be used to conclude that these techniques can safely protect postoperative hearing.

Perhaps, in future *Annals* publications, some standard guidelines for animal ABR stimuli and calibration could be used, so that possible future patient care will not be based on potentially faulty animal data.

Kathleen Campbell, PhD
Southern Illinois University School of Medicine
Springfield, IL 62794-9230

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FLASH PUMP DYE LASER TREATMENT OF LARYNGEAL PAPILLOMAS

CHARLES M. BOWER, MD
STEPHEN FLOCK, PHD
MILTON WANER, MD
ROBERT SCHAEFFER, MD

LITTLE ROCK, ARKANSAS

To evaluate the feasibility and safety of the flash pump dye (FPD) laser for the treatment of laryngeal papillomas, we performed a prospective nonrandomized trial comparing FPD and carbon dioxide (CO2) laser treatment of laryngeal papillomas in a tertiary care children's hospital. Nine patients from 2 to 20 years of age with severe recurrent laryngeal papillomas were enrolled in the study. All patients underwent CO2 laser debulking of papillomas on the left hemilarynx, with 8 W continuous or pulsed energy. The right hemilarynx was treated with the FPD laser with 12 to 77 pulses at 8 to 12 J, through a custom-designed 90° firing fiber. An end point of blanching and purpura of the papillomas was used. The main outcome measures were the safety and ease of operation with the FPD laser, and decreased papilloma size based on visual inspection at 2 weeks postoperative and at the next laser procedure. Seven patients were irradiated 1 time with an FPD laser, and 2 patients 2 times. No intraoperative complications were noted. One patient developed early postoperative stridor. No patients described more discomfort, and 5 patients described their voice as being the same as or better than it was after prior CO2 laser procedures. Five patients had a 90% or more decrease in size of papillomas on the FPD-irradiated side 2 weeks postoperatively. Three patients had approximately a 50% reduction. A treatment effect was noted in all patients, and was similar to the results noted on the CO2 laser–treated side. Early results with FPD laser treatment of laryngeal papillomas suggest the protocol is relatively safe and feasible. Long-term results are pending. Because the FPD laser coagulates rather than vaporizing tissue, potential advantages may include decreased scarring relative to CO2 laser treatment, and improved patient and operator safety.

KEY WORDS — flash pump dye laser, human papillomavirus, laryngeal papilloma.

INTRODUCTION

Laryngeal papillomas are benign superficial tumors of the larynx that result from human papillomavirus (HPV) infection of the larynx. Laryngeal papillomas are the most common laryngeal mass in children, and cause significant morbidity consisting of hoarseness, dyspnea, and potential for airway compromise and death. Dissemination of papillomas to the trachea and lungs can cause life-threatening airway obstruction or pulmonary compromise. There is also a suspected relationship between HPV and malignant tumors of the larynx.[1,2]

Unfortunately, there is no curative therapy for laryngeal papillomas, and the current therapy of choice involves removing obstructing, symptomatic lesions with the carbon dioxide (CO2) laser.[3-5] Intermittent removal is required at intervals ranging from every few weeks to 6 months or a year. Complications may arise from repeated surgery due to the concomitant thermal damage to adjacent uninvolved tissue with resulting scarring, or spread of the infection either distally or to operating room personnel from the smoke plume from ablated tissue.[6-14]

Lasers that produce pulsed yellow light have been used for a number of years in treating a variety of benign cutaneous lesions.[15] Pulsed dye lasers such as the flash lamp–pumped dye (flash pump dye; FPD) laser produce excellent clinical results by selectively coagulating abnormal vascular tissue while at the same time sparing uninvolved tissue.[16] Yellow light at or near 577 nm, produced by the FPD laser, is strongly absorbed by hemoglobin, and less absorbed by other tissues; this phenomenon results in more selective photothermolysis in vascular lesions.[17,18] Laryngeal papillomas may be amenable to treatment with the FPD laser because of documented vascularity that could strongly absorb laser light, resulting in photothermolysis. In vivo demonstration of a prominent vascular loop within each papilla,[19] and histologic demonstration of the presence of dilated, congested blood vessels in papillae of papillomas support this theory.[20] Recent data have demonstrated early effectiveness of FPD laser treatment of cutaneous verrucae, with 72% of patients cleared of warts after 2 treatments.[21] It was therefore hypothesized that FPD laser treatment of laryngeal papillomas could result in short- or long-term control of laryn-

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From the Department of Otolaryngology–Head and Neck Surgery, Arkansas Children's Hospital (Bower, Waner, Flock), and the Department of Otolaryngology, Veterans Administration Hospital of Little Rock (Schaeffer), Little Rock, Arkansas.

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CORRESPONDENCE — Charles M. Bower, MD, Division of Otolaryngology, Arkansas Children's Hospital, 800 Marshall St, Little Rock, AR 72202.

geal papillomas.

The purpose of this preliminary study was to investigate whether the FPD laser is potentially useful for the treatment of laryngeal papillomas. The FPD laser ablation of tissue may result in more complete destruction of papilloma tissue, and thus conceivably increased time between local recurrences. Because the FPD laser coagulates rather than ablating tissue, less scarring is likely. Also, a visible plume of infected tissue is not produced; thus, the possibility of iatrogenic spread of disease distally or to operating room personnel is essentially eliminated. Finally, the ability of FPD laser energy to be delivered fiberoptically opens up new possibilities for treatment, such as outpatient fiberoptic ablation of papillomas.

MATERIALS AND METHODS

Children older than 1 year of age with recurrent laryngeal papillomas were candidates for the present study. All patients required at least 3 laser treatments prior to the first FPD treatment. Exclusion criteria were age under 1 year, complicating medical conditions such as asthma, congenital heart disease, seizures, or cerebral palsy, and lack of informed consent.

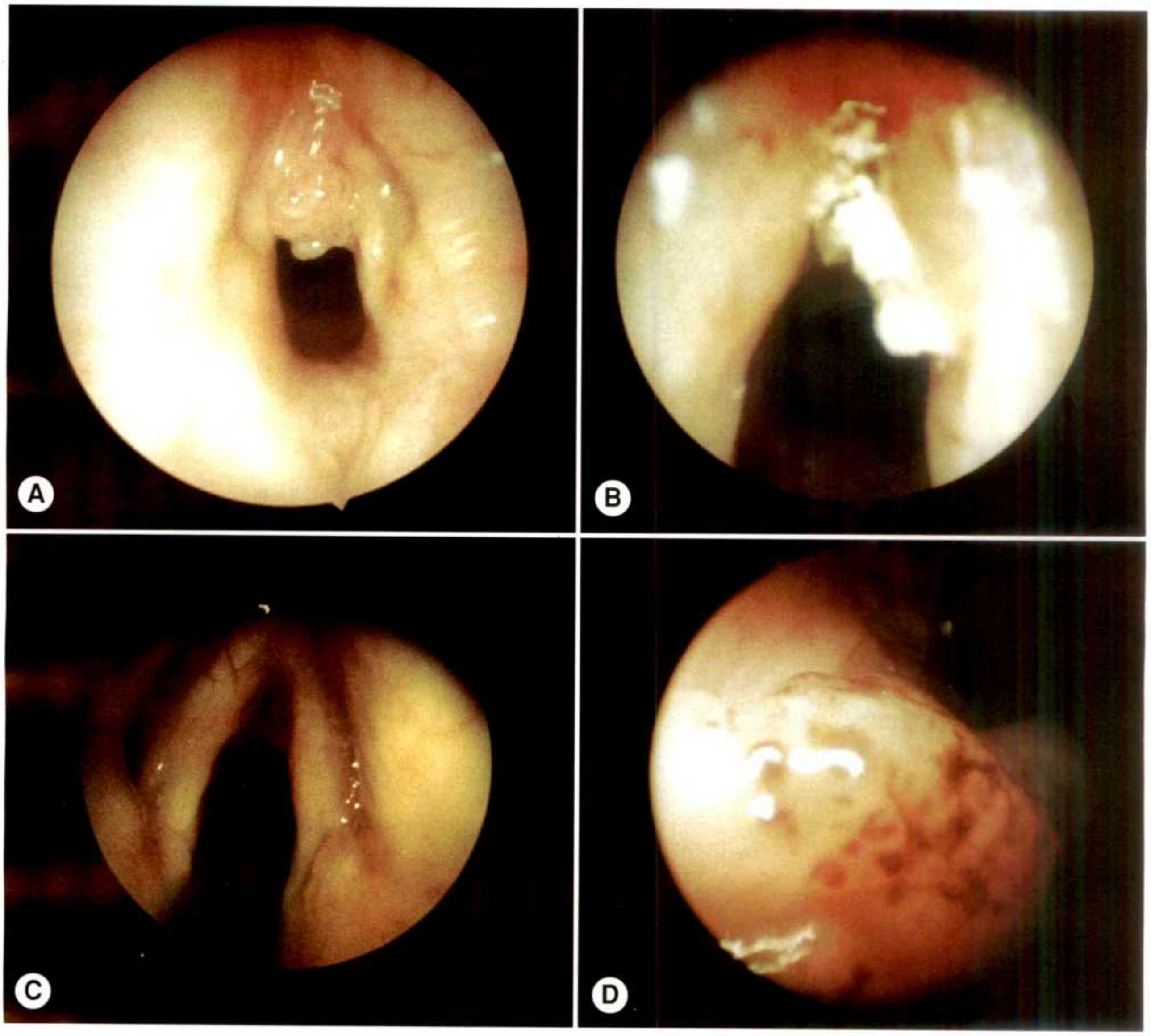
After informed consent was obtained, patients were taken to the operating room, where they were placed under general masked anesthesia. The larynx was topically anesthetized with lidocaine hydrochloride, and an appropriate laryngoscope and a Hopkins rod telescope were used for evaluation. Photographic documentation was obtained of the larynx and trachea prior to and subsequent to each intervention (see Figure, A-C). Direct laryngoscopy was performed, and a CO_2 laser (Sharplan model Surgilase 60XJ) was used to remove the laryngeal papillomas from the supraglottic larynx and the left true vocal cord. Care was taken to avoid the anterior commissure. Patients were left breathing spontaneously throughout this procedure.

A consistent area of papillomas was left untreated by the CO_2 laser along the right vocal cord. All patients had disease in this area. If any bleeding was apparent, this was stopped with topical oxymetazoline hydrochloride on a pledget. An FPD laser (Cynosure Photogenica V) was initially used at a setting of 8 J to remove the study papillomas. After we completed an animal protocol demonstrating relative safety of higher power settings, a dosage of 12 J/cm^2 was used for the remaining 5 patients. The laser light was delivered through a specially designed fiber with a prism on the end allowing 90° irradiation at the tip of the probe. The laser was set to irradiate the larynx at a distance of approximately 2 to 3 mm, depending on patient size, and with a spot size of approximately 2 × 3 mm. The fiber was passed down through the laryngoscope and positioned to irradiate the residual papillomas. Eye protection for the operating surgeon and operating room personnel was required. The residual papilloma was irradiated with 12 to 77 bursts to treat the entire lesion. An end point of significant purpura and blanching of the residual papillomas was used (see Figure, D). Flexible nasopharyngoscopy was performed on postoperative day 1 on the first 2 patients, and on postoperative day 14 on all patients. Response to treatment was subjective and based on endoscopic inspection of the larynx. Response was estimated as percent reduction in size, then categorized as no response, more than 50% reduction in size, or more than 90% reduction as compared to intraoperative photographs. All patients returned to the operating room for follow-up endoscopy and laser as per their routine treatment protocol. The Institutional Review Board approved this study prior to enrolling any patients.

RESULTS

Nine patients, ages 2 to 20 years (average 10 years), were enrolled in the protocol. Four were male and 5 were female. In the year prior to the study, the patients had undergone surgical excision of papillomas from 0 to 9 times (average 4.6). All but 1 patient returned for follow-up visits. Duration of follow-up from the first FPD treatment ranged from 2 weeks (ie, lost to follow-up) to 19 months (average 7 months). Seven patients were irradiated 1 time with an FPD laser, and 2 patients 2 times. All patients were FPD laser–treated to an end point of purpura and blanching. The Figure, A-C, demonstrates the intraoperative effects of the FPD laser. No intraoperative complications were noted.

One patient developed early postoperative stridor requiring Vaponephrine updrafts and an intravenous dose of dexamethasone sodium phosphate. The patient's stridor resolved that afternoon, and he was discharged the next day with no complications. Eight patients described the procedure as being essentially the same in terms of discomfort relative to other procedures. None described more discomfort. Five patients described their voice as being the same as or better than it was after prior CO_2 laser procedures, while 2 complained of a somewhat worse voice immediately postoperatively. No patients complained of increased difficulty breathing or earlier onset of dyspnea after the FPD laser treatment relative to other treatments.



Endoscopic views of glottis. A) Demonstrating papillomas in anterior commissure region and along anterior true vocal cords prior to treatment. B) Intraoperative view of same patient, demonstrating blanching and desiccation of tissue treated with flash pump dye laser. C) Same patient, now demonstrating significant decrease in size of papillomas at 6-month follow-up endoscopy. D) Close-up endoscopic view of laryngeal papillomas in another patient, partially treated with flash pump dye laser, demonstrating purpuric change of each central vessel.

One-day follow-up endoscopy was performed on the first 2 patients. Blanching and modest involution of the treated papillomas was noted, but no significant edema or surrounding erythema. A 2-week follow-up endoscopy was performed on all but 1 patient, who was lost to follow-up. Five patients had a 90% or more decrease in size of papillomas on the irradiated side 2 weeks postoperatively. Three patients had approximately a 50% reduction. A treatment effect was noted in all patients, and was similar to the results noted on the CO_2 laser–treated side.

In this brief study, no change was noted in the frequency of need for reoperation for recurrent disease. Significant scarring was not noted in this preliminary study, but long-term results are not available. That no plume of smoke was noted in any patients suggests a low risk for iatrogenic spread of disease.

DISCUSSION

Carbon dioxide laser ablation of laryngeal papillomas remains the mainstay of treatment for this recurring and quite debilitating disease. The CO_2 laser has the advantage of an immediate effect in reducing the size of bulky disease, resulting in immediate improvement in the airway that allows for an early discharge for most patients. Unfortunately, repeated procedures are often necessary, as excision does not cure the underlying disease. There is also a significant risk of scarring, which has been reported in up to 45% of patients.[3,4]

Because of the inadequacy of repeated laser excisions, a multitude of other treatments have been tried, including cryosurgery, cauterization, electrocautery, photodynamic therapy, and a variety of medications, including estradiol, steroids, antibiotics, and a variety of chemotherapeutic agents. Most recently, acyclovir, isotretinoin, and interferon have been used with some degree of success.[22-28] Use of other lasers has been tried in the treatment of papillomas, including blue-green argon lasers and contact-tip neodymium:yttrium-aluminum-garnet lasers, but has not been pursued because of inadequate effect or significant scarring.[29]

Because of the potential disadvantages of the CO_2 laser, including repeated excisions, the potential for scarring, and the potential for iatrogenic spread of disease from an infected plume of smoke, we felt investigation of alternative treatment was appropriate. We hypothesized the FPD laser might be a potential clinical alternative for treatment of laryngeal papillomas. The yellow light of the FPD laser has a wavelength of 577 nm that is strongly absorbed by hemoglobin and less absorbed by other molecular species. These lasers have been used since 1983 to treat vascular lesions not amenable to other forms of therapy, with very good success. As contact microscopic evaluation of papillomas revealed a prominent vascular loop in the center of each papilla,[19] it was theorized that the FPD laser could result in coagulation necrosis of papillomas. Since this vascular loop appears more prominent than surrounding submucosal vessels, presumably, coagulation of papillomas could occur with less treatment-induced scarring of surrounding normal tissue. The FPD laser treatment of cutaneous verrucae has at least some clinical benefit.

In this pilot study, the FPD laser was used to treat a nonobstructing area of papillomas in children's larynges. The study was designed to demonstrate safety and feasibility. This study did not prove effectiveness of opening the airway in children with significant obstructing lesions, as all children had concomitant CO_2 laser treatment for maintenance of the airway. In fact, at 24 hours after irradiation of the papillomas, little change in the airway was noted. The study did, however, demonstrate that the FPD laser could be used on smaller lesions to relatively safely coagulate the papillomas, with significantly decreased tumor size at 2 weeks. Early postoperative stridor was noted in 1 patient, but resolved with minimal intervention. No other intraoperative or postoperative complications were noted. While decreased scarring is a theoretic advantage, and no early scarring was appreciated, this pilot study with short-term follow-up was unable to fully assess this potential advantage of the FPD laser.

Reduction in at least 50% of the size of the papillomas with moderate doses of FPD laser light was noted in 8 patients, and greater than 90% reduction was noted in 5 of the 8 patients. Unfortunately, the early, 2-week results have not yet translated into a more durable treatment in terms of less frequent need for recurring therapy.

While the FPD laser has certain theoretic advantages in treatment of patients with laryngeal papillomas, it is unlikely, except in patients with fairly minimal disease, that this laser will be able to be used exclusively to treat patients with recurrent laryngeal papillomatosis. While effectiveness and relative safety have been demonstrated, this treatment is for limited sites of disease only. It is certainly possible that treating high-risk areas such as the anterior and posterior commissures with the FPD laser may provide a significantly improved voice quality, a better airway, and a longer interval between treatments without the potential risks of scarring as compared to the CO_2 laser. It is also certainly likely that the risk of iatrogenic spread with the FPD laser is considerably less. Using the laser under local anesthetic delivered through a flexible bronchoscope may also be possible.

SUMMARY

1. Early results with FPD laser treatment for laryngeal papillomas suggest the protocol is relatively safe and feasible.

2. Because the FPD laser coagulates tissue, rapid removal of obstructing or bulky disease is not possible; this limitation precludes sole use of the FPD laser for patients with severe disease.

3. Reduction in papilloma size of greater than 90% in treated areas was noted in 5 patients, and greater than 50% in 3 other patients.

4. Because the FPD laser coagulates rather than vaporizing tissue, potential advantages include decreased scarring relative to CO_2 laser treatment, and improved operator and patient safety because a potentially infectious plume of smoke is not produced. The FPD laser may be particularly advantageous for papillomas in high-risk areas such as the anterior commissure of the larynx.

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USE OF MONTGOMERY® T-TUBE IN LARYNGOTRACHEAL RECONSTRUCTION IN CHILDREN: IS IT SAFE?

YORAM STERN, MD
PETAH-TIKVA, ISRAEL

J. PAUL WILLGING, MD
CINCINNATI, OHIO

ROBIN T. COTTON, MD
CINCINNATI, OHIO

Montgomery® T-tubes are often used in adult patients; however, they are less commonly used in the pediatric age group. The purpose of this report is to describe our experience with this stent in pediatric laryngotracheal reconstruction. A retrospective chart review was performed to identify early and late complications. Nutritional assessments were made on the basis of weight percentile comparisons at the time of surgery and at the end of the stenting period. From 1990 to 1997, the Montgomery® T-tube was utilized in 26 children undergoing 36 airway reconstruction procedures (21 laryngotracheoplasties and 15 cricotracheal resections). The upper limb of the tube extended above the level of the glottis in all patients. The patients ranged in age from 2.4 to 17.9 years. The duration of stenting ranged from 2 weeks to 23 months. Three patients (11.5%) had significant aspiration and did not improve following diet modifications and swallowing therapy, requiring tube feedings. One patient had postoperative subcutaneous emphysema that resolved spontaneously. Three patients required early removal of the stent due to an inability to tolerate plugging. Granulation tissue above the upper limb of the stent during the stenting period was noted after 6 laryngotracheal reconstruction procedures (16%). Only 1 patient fell off his growth curves during the period of stenting. There were no deaths in this series, and no emergent procedures were required. Postoperative and home care and management of complications are discussed. Our experience indicates that Montgomery® T-tubes can be utilized relatively safely in children, providing that postoperative and home care are meticulous.

KEY WORDS — children, laryngotracheal reconstruction, T-tube.

INTRODUCTION

The use of intraluminal stents has become an integral part of laryngotracheal reconstruction (LTR). The purposes of an intraluminal stent are to hold grafts or flaps in position, support the reconstructed but still unstable laryngotracheal framework, and prevent scar contracture.[1] Safety of use has played an important role in this process. Commonly used stents include rolled silicone sheeting ("Swiss roll"),[2] the Polytef tube (Aboulker, Cotton-Lorenz),[3] and preformed hollow silicone (Montgomery® T-tube).[4] The Montgomery® T-tube has been used mainly in adult patients, with low complication rates and good results.[5,6] However, because of the decreased inner diameter of the pediatric-size T-tubes, they are not often used in children.

The objective of the present report is to present our experience with the use of the Montgomery® T-tube in LTR in children, with special emphasis on safety.

SUBJECTS AND METHODS

Subjects. All the pediatric patients (age < 18 years) who underwent LTR with use of a Montgomery® T-tube at the Department of Pediatric Otolaryngology, Children's Hospital Medical Center, Cincinnati, Ohio, between July 1990 and July 1997 were included in the study. The patient's charts were reviewed with respect to the following variables: age, sex, type of airway surgery, T-tube size, early postoperative complications, length of postoperative hospital stay, length of stenting, emergency room visits and hospitalization during the period of stenting, and late complications. Nutritional assessments were made on the basis of weight percentile comparisons at the time of surgery and at the end of the stenting period.

Surgical Considerations. In all the patients, the end of the upper intraluminal limb of the tube extended above the glottis. The end of the lower intraluminal limb was positioned at least 5 mm above the carina. The intraluminal ends were trimmed as necessary, and the customized ends were beveled and smoothed to prevent airway injury. Prior to insertion, the distances between the tip of the extraluminal limb and the ends of the intraluminal limbs were carefully measured and recorded. These were used as references for later suctioning. The proper position

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From the Department of Pediatric Otolaryngology and Maxillofacial Surgery, Children's Hospital Medical Center and University of Cincinnati College of Medicine, Cincinnati, Ohio.

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CORRESPONDENCE — Yoram Stern, MD, Dept of Pediatric Otolaryngology, Schneider Children's Medical Center of Israel, 14 Kaplan St, Petah-Tikva 49202, Israel.

AIRWAY PROCEDURES WITH USE OF MONTGOMERY® T-TUBE (N = 36)

Procedure	*No.*
Cricotracheal resection	15
Anterior split	10
Anterior split and buccal mucosal graft	4
Anterior and posterior splits	4
Anterior and posterior splits with cartilage grafts	2
Anterior split with cartilage graft	1

of the T-tubes was checked endoscopically intraoperatively. At the time of final removal of the T-tube, small horizontal skin incisions were made at both sides of the external limb, and the anterior aspect of the airway around the T-tube was exposed. This ensured relatively safe removal of the T-tube and insertion of a tracheostomy tube.

Postoperative Care. As soon as the patients awoke from the general anesthesia, the T-tube was plugged. Suctioning the T-tube after lavage with normal saline was performed every 4 hours in the first 24 postoperative hours, and then every 8 hours or as necessary. Sodium bicarbonate solution (5%, 2 mL) was used to loosen thick secretions. For emergent removal of the T-tube, a hemostat and tracheotomy tube (of smaller size than the T-tube) with obturator were taped to the head of the patient's bed and provided to his or her caregivers on discharge home. All the patients were discharged from the hospital only when they were able to tolerate complete plugging of their T-tube and did not aspirate and their caregivers were educated and experienced with the special care of the T-tube. During the period of stenting, fiberoptic examination of the larynx was usually done every 4 weeks, or if the patient did not tolerate plugging of the T-tube, to ensure proper placement of the tube.

Statistical Analysis. Statistical analysis was done with SAS 6.8 statistical software (SAS Institute, Cary, NC). Kendall's correlation coefficient was used to calculate significant associations between variables (age, type of airway surgery, T-tube size, and length of stenting) and outcomes (postoperative hospital stay, complications, and change in weight percentile).

RESULTS

From July 1990 to July 1997, Montgomery® T-tubes were utilized in 26 children who underwent 36 airway reconstruction procedures (see Table). The patients included 19 boys and 7 girls, and their ages at the time of surgery ranged from 2.4 to 17.9 years (mean ± SD, 8.4 ± 3.7 years). The sizes of the T-tubes used ranged from 6 mm (outside diameter) to 12 mm (Fig 1). The 8-mm size was most commonly used; the 6-mm T-tube was used only in 1 patient. One patient had postoperative cervical subcutaneous emphysema that resolved spontaneously. Six patients (23%) had significant postoperative aspiration. Three of these patients did not improve following diet modifications and swallowing therapy, requiring tube feeding. Five of the 6 patients who had significant postoperative aspiration, and all the patients who required tube feeding, underwent cricotracheal resection. The association between cricotracheal resection and the need for tube feeding was statistically significant ($p < .01$). Three patients (11.5%) did not tolerate plugging of the T-tube, and early removal of the tubes was required. The postoperative hospital stay ranged from 2 to 21 days (mean ± SD, 6.9 ± 6.0 days). Patients who underwent cricotracheal resection usually had a longer hospital stay than patients who underwent other airway surgeries, although this difference was not statistically significant ($p = .06$). The duration of the stenting period ranged from 2 weeks to 23 months (mean ± SD, 7.4 ± 6.3 months). Formation of granulation tissue above the upper limb of the T-tube was noted during the stenting period after 6 LTR procedures (Fig 2). The position of the upper limb of the T-tube was not high-

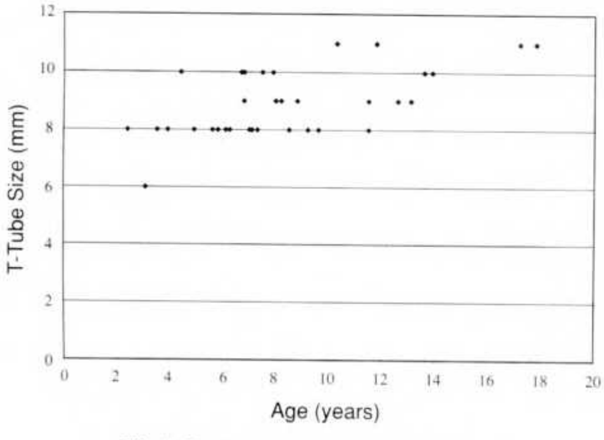

Fig 1. T-tube sizes according to age.

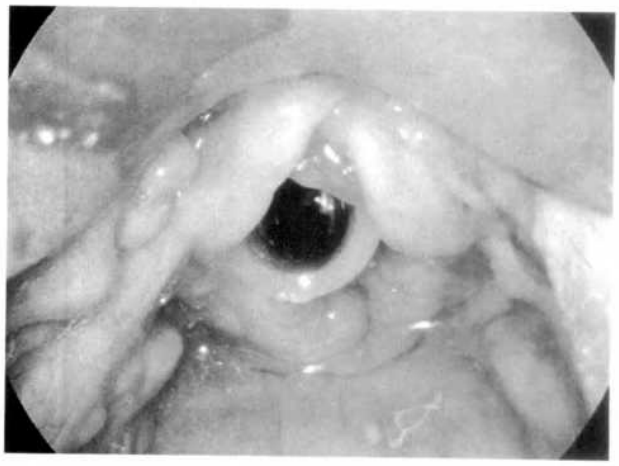

Fig 2. Granulation tissue above upper limb of T-tube.

er in these cases. No significant formation of granulation tissue was noted below the lower limb of the T-tube. There were no deaths or emergent procedures during the period of stenting in this series. During the stenting period, only 1 patient fell off his growth curve (from 90th percentile to 50th percentile), while all other patients maintained or increased their growth curves. At the end of the stenting period, the T-tube was removed without difficulty in all of the patients.

DISCUSSION

There are many factors impacting upon the success of LTR, including the cause of the stenosis, the degree of stenosis, and the surgical technique. Stenting has been advocated for use in cases of moderate to severe stenosis involving the supraglottis, glottis, subglottis, or trachea.[7] The ideal laryngotracheal stent must conform to the lumen, be flexible, exert gentle pressure on the mucosal surface, and be stable within the lumen. It must be easily removed and replaced, avoid aspiration, and allow voice and respiration.[7,8] The variety of stents in use reflects the fact that no single stent is ideal. The Montgomery® T-tube has the advantage of being both a stent and a tracheotomy tube.[9] It is made of silicone, which causes little tissue reaction and is sufficiently soft so as not to injure the lining or supporting structure of the larynx and the trachea. It is self-secured, is easy to replace or remove, and allows normal respiration and speech. However, the Montgomery® T-tube has a potential risk of obstruction, especially when the inner diameter is small. T-tube obstruction was not noted in our series. However, most of the T-tubes used in our series were of a size equal to or larger than 8 mm, and only 1 small (6 mm) T-tube was used. Failure to maintain plugging of the T-tube or inadequate suctioning may result in its obstruction due to accumulation of mucous and viscous secretions. In our experience, this risk can be minimized by continuous plugging of the tube and proper suctioning. If the patient does not tolerate plugging of the T-tube, early removal of the stent should be considered. The patient's family and caregivers need to be aware of the possible risk of obstruction and should be adequately trained and equipped for emergent removal of the tube and replacing it with a tracheotomy tube.

All patients in our series had subglottic lesions and needed subglottic stenting. The T-tube was placed through both the true and false vocal cords in order to prevent severe scarring and formation of granulation tissue in the glottis. Placement of the tube in the proper location in the larynx is very important; if it is placed higher than the level of the arytenoids, aspiration is almost inevitable. Therefore, the position of the tube should be checked endoscopically, both intraoperatively and postoperatively. Long-term aspiration was not noted in most of the patients who underwent laryngotracheoplasty procedures, as was also reported by other authors in adult patients.[10] Patients who underwent cricotracheal resection were more prone to develop postoperative aspiration. The relatively high incidence of aspiration in these patients may be related to some surgical features of cricotracheal resection, such as the suprahyoid release that is done as part of the procedure and the flexion sutures ("chin to chest") that were used postoperatively.[11] Therefore, patients who undergo cricotracheal resection with insertion of T-tubes should be carefully observed for any sign of aspiration, including recurrent lower airway infection. When aspiration is noted, diet modifications and swallowing therapy should be initiated. If no improvement is observed, tube feeding should be recommended.

Although increased formation of granulation tissue with use of the Montgomery® T-tube has been reported,[12] it was observed in a relatively low percentage (16%) of procedures in our series. Moreover, no formation of tracheal granulation tissue was noted below the lower end of the T-tube. This is in contrast to the use of the short Aboulker stent, which often causes granulation tissue formation at the lower end of the stent, leading to possible circumferential tracheal stenosis.[8] Proper placement of the upper tip of the T-tube above the glottic level and beveling the ends after adjusting the tube lengths is important in preventing laryngeal and tracheal injury and granulation tissue formation.

CONCLUSION

Our experience indicates that Montgomery® T-tubes can be utilized relatively safely in children undergoing LTR. Patients undergoing cricotracheal resection are at greater risk for postoperative aspiration and should be carefully observed for this possible complication. Meticulous intraoperative adjustment and postoperative and home care are of great importance in maintaining safety and avoiding complications.

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EVALUATION OF GASTROESOPHAGEAL REFLUX IN PATIENTS WITH LARYNGOTRACHEAL STENOSIS

ROBERT J. TOOHILL, MD
SECKIN O. ULUALP, MD
REZA SHAKER, MD
MILWAUKEE, WISCONSIN

To report the incidence of pharyngeal acid reflux events in patients with laryngotracheal stenosis (LTS), we studied 12 patients with LTS and 34 healthy volunteers. All patients and controls underwent ambulatory 24-hour 3-site pH monitoring. In ambulatory pH monitoring, pH was recorded at manometrically determined sites of the pharynx, proximal esophagus, and distal esophagus. For all 3 sites, a pH value below 4 that was not related to the time of oral intake or belching was considered an acid reflux event. Eight of the 12 LTS patients exhibited pharyngeal acid reflux events. In the control group, pharyngeal acid reflux events were documented in 7 subjects. In between-group comparison, the number of reflux episodes and the percent acid exposure time in the pharynx were greater in LTS patients than in controls. Reflux parameters of the proximal and distal esophagus in LTS patients were similar to those of controls. The incidence of pharyngeal acid reflux events in LTS patients was higher than that in controls. It is suggested that identification and treatment of gastroesophageal reflux in patients will significantly simplify and improve the results of treatment for LTS.

KEY WORDS — gastroesophageal reflux, laryngotracheal stenosis, pharyngeal acid reflux.

INTRODUCTION

A recent significant direction in understanding the cause and treatment of laryngotracheal stenosis (LTS) has been the suggestion that gastroesophageal reflux disease (GERD) is an associated factor in the pathogenesis.[1]

Bogdasarian and Olson[2] first suggested that gastric acid reflux might play a significant role in the cause of posterior glottic stenosis. Bain et al,[3] in 1983, reported a case of subglottic stenosis (SGS) in a 57-year-old woman with no previous history of surgery, trauma, or intubation. Her preoperative airway was described as being "pencil eraser size," and 5 months after a Nissen fundoplication and endoscopic laryngeal repair, her airway was normal. Little et al,[4] in 1985, reported the case of a 12-year-old girl with recalcitrant SGS who continued to produce exuberant granulation tissue. During an endoscopic procedure, she was noted to have gastric contents in her hypopharynx and larynx. The patient was treated with H2 blockers and reflux precautions and was subsequently decannulated, with cessation of all further granulation tissue formation.

The largest study to date involving GERD and LTS was conducted by Koufman[1] in 1991. He studied 32 patients with tracheal stenosis and SGS with a 24-hour intraesophageal pH monitor and demonstrated significant GERD with pharyngeal acid reflux in three fourths of his patients. Jindal et al[5] and Toohill and Jindal[6] reported studies of 9 adult women with idiopathic SGS that was refractory to all methods of management. When GERD was recognized in these patients and subsequently treated, the management of their SGS was much more successful. Burton et al[7] reported this relationship in pediatric SGS and urged aggressive medical management. Two reports of idiopathic SGS did not consider GERD as a possible etiologic factor.[8,9] Toohill[10] reported excellent results in the management of all cases of LTS when they were concomitantly managed with antireflux treatment.

There is continued controversy over the relationship of GERD to the cause and treatment of LTS. Since Koufman's[1] 1991 study, there have been no additional studies to investigate this relationship. Recent development of improved 3-site 24-hour pH monitoring techniques with pharyngeal measurement of reflux has given additional information in supraesophageal complications of GERD.[11] In the present study, we utilized this technique in 12 consecutive patients presenting with LTS.

METHODS

Twelve patients with LTS (7 male, 5 female), ages

From the Medical College of Wisconsin Dysphagia Institute (all authors), the Department of Otolaryngology and Communication Sciences (all authors), and the Division of Gastroenterology and Hepatology (Ulualp, Shaker), The Medical College of Wisconsin, Milwaukee, Wisconsin.

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CORRESPONDENCE — Robert J. Toohill, MD, Dept of Otolaryngology and Communication Sciences, Froedtert Memorial Lutheran Hospital, 9200 W Wisconsin Ave, Milwaukee, WI 53226.

TABLE 1. DEMOGRAPHICS OF LARYNGOTRACHEAL STENOSIS PATIENTS

Patient	*Sex*	*Age*	*Site of Stenosis*	*Cause*
1	M	68	Subglottic	Intubation
2	F	55	Subglottic	Idiopathic
3	F	57	Glottic	Intubation
4	M	22	Glottic	Intubation
5	M	63	Glottic	Intubation
6	M	37	Glottic	Intubation
7	M	73	Glottic	Irradiation, cancer
8	F	77	Glottic, tracheal	Arytenoid fixation from rheumatoid arthritis
9	M	38	Subglottic, tracheal	Intubation
10	F	50	Subglottic, tracheal	Idiopathic
11	F	52	Glottic, subglottic	Idiopathic
12	M	30	Glottic	Intubation

from 22 to 77 years (52 ± 5 years), and 34 healthy volunteers (19 male, 15 female), ages from 19 to 85 years (39 ± 4 years), were studied. Studies were approved by the Human Research Review Committee of The Medical College of Wisconsin, and participants gave written informed consent before their studies. All patients and healthy volunteers filled out a detailed health questionnaire before their studies.

Normal healthy volunteers were recruited by advertisement and did not have any esophageal or laryngeal symptoms. In addition, they underwent unsedated transnasal pharyngoesophagogastroduodenoscopy and did not exhibit any disorders.

Laryngotracheal stenosis patients complained of dyspnea, frequent throat clearing, or chronic hoarseness. All patients exhibited posterior laryngitis findings on stroboscopic examination. The causes and sites of stenosis for each patient are tabulated in Table 1.

All patients and normal volunteers were studied for occurrence of pharyngeal acid reflux by 3-site 24-hour pharyngoesophageal pH monitoring.

The ambulatory pH studies were done with two pH recording systems: one with a single recording site, and another with 2 recording sites that were 10 cm apart (Synectics Medical Inc, Irving, Tex). The pH probes were placed at 3 manometrically determined sites: the pharynx (2 cm above the upper esophageal sphincter); the proximal esophagus (10 cm distal to pharyngeal probe, approximately 4 to 5 cm below the upper esophageal sphincter); and the distal esophagus (5 cm above the lower esophageal sphincter). The probes were secured to the nose. Studies were initiated on the morning after the manometric studies and terminated 22 to 24 hours later. Subjects ate standard meals that included the following: breakfast, a total of 504 kcal (79% carbohydrate, 12% protein, 9% fat); lunch, a total of 822 kcal (68% carbohydrate, 11% protein, 21% fat); and dinner, a total of 1,199 kcal (58% carbohydrate, 16% protein, 26% fat). Meals were provided through the Medical College of Wisconsin General Clinical Research Center. All subjects kept a detailed diary indicating the times of oral intake and time of retiring. They also indicated position (upright or supine), and events such as heartburn, belching, cough, chest pain, and exercise. For all 3 sites, a decrease in pH below 4 that was not related to belching or to the time of eating or drinking was considered an acid reflux episode. For the pharyngeal site, it had to be simultaneous with or preceded by a decrease in pH of similar or larger magnitude in the proximal and distal esophageal sites to be accepted as a pharyngeal reflux event. Earlier studies have shown that the proximal distribution of refluxate is associated with a decline in pH of refluxed material in the esophagus. Also, determination of the temporal relationship between the onset of pH decline among recording sites differentiated pH declines induced by oral intake (in which pharyngeal pH decline precedes distal esophageal pH drops) from true gastroesophagopharyngeal acid reflux episodes (in which pharyngeal pH decline either occurs simultaneously with or after the esophageal pH decline). These strict criteria were applied to avoid counting spurious readings induced by pharyngeal pH probe movement, loss of complete mucosal contact, pH change due to aerodigestive tract residue and secretions, and pH change due to oral intake.[11]

During the study, signals from pH electrodes were stored by the portable data logger, and at the completion of each study, data were transferred to a computer for subsequent printing and analysis. In addition, pH recordings were displayed on the screen by using the capability of the program to use a smaller time scale for determination of the temporal relationship among pH declines registered at various sites. The 2 pH electrodes were calibrated in buffers of pH 1 and pH 7, before and at the end of each study, and showed no significant drift in the pH signal during the study. Using these techniques, we determined the number of pH declines below 4, and the percent of study time that the pH was below 4 in the pharynx, proximal esophagus, and distal esophagus. Percent acid exposure time was calculated as percentage of study period that the pH sensor was exposed to acid. Statistical comparisons between groups for

TABLE 2. FINDINGS OF AMBULATORY 24-HOUR 3-SITE pH MONITORING IN PHARYNX

Patient	*No. of Reflux Events*	*% Acid Exposure Time*
1	4	0.12
2	1	0.01
3	1	0.008
4	12	0.62
5	0	0
6	2	0.54
7	1	0.008
8	0	0
9	0	0
10	5	0.11
11	0	0
12	3	0.06

TABLE 3. COMPARISON OF FINDINGS OF AMBULATORY 24-HOUR 3-SITE pH MONITORING

	Patients (n = 12)	*Controls (n = 34)*
Pharynx		
No. of episodes	1*	0
% Acid exposure time	0.009*	0
Proximal esophagus		
No. of episodes	11.5	7
% Acid exposure time	0.9	0.4
Distal esophagus		
No. of episodes	34.5	25
% Acid exposure time	5	2.6

Data are median values.
*p < .05.

number of acid reflux events and acid exposure time were performed with a Mann Whitney rank sum test, and for the prevalence of pharyngeal acid reflux events, Fisher's exact test was performed.

RESULTS

Pharynx. Seven of the 34 normal controls exhibited pharyngeal acid reflux events. All pharyngeal acid reflux events occurred in the upright position. The number of pharyngeal acid reflux events ranged from 0 to 3. In the LTS group, 8 patients exhibited pharyngeal acid reflux events. All 8 patients exhibited pharyngeal acid reflux events in the upright position. The number of pharyngeal acid reflux events ranged from 0 to 12 among LTS patients (Table 2). In the LTS group, pharyngeal acid reflux events were documented in significantly more subjects than in the control group ($p < .05$). In between-group comparison, the number of pharyngeal acid reflux events in LTS patients was greater than that in normal controls ($p < .05$; Table 3). Similar to the number of pharyngeal acid reflux events, LTS patients had a greater percent acid exposure time compared to normal controls ($p < .05$; Table 2).

Proximal Esophagus. Four of the 34 normal controls did not exhibit any proximal esophageal acid reflux events. The number of proximal esophageal acid reflux events ranged between 0 and 87 in the control group.

In the LTS group, 11 of the 12 patients exhibited proximal esophageal acid reflux events. The number of proximal esophageal reflux events per patient was between 0 and 40 in the LTS group. Neither the number of events nor the percent acid exposure time in the proximal esophageal site was significantly different between LTS patients and normal controls (Table 3).

Distal Esophagus. All subjects exhibited distal esophageal reflux events except 2 normal controls. The number of distal esophageal reflux events ranged from 0 to 121 in the control group and 11 to 69 in the LTS group. Similar to the proximal esophagus between-group comparison, both the number of reflux events and the percent acid exposure time in the distal esophagus were similar between LTS patients and normal controls (Table 3).

DISCUSSION

The impetus for this study was based on suggestions from recent studies.[1-7,10] There are other considerations that add credence to the suggestion that GERD plays a role in the development of LTS. Many of the patients who develop LTS have an associated severe trauma or illness that requires intubation. The effect on the lower esophageal sphincter in critical illness or anesthesia has been well documented.[12] Patients with these morbid conditions have an inability to swallow, which to a large extent prohibits the peristaltic propulsion of gastric contents back into the stomach. This factor, along with the recumbent position of the seriously ill patient, promotes "silent" reflux in the hypopharynx and penetration and aspiration in the larynx and trachea. Many medications used for critically ill patients likewise contribute to dysfunction of the lower esophageal sphincter and reflux.[7] A modest lowering of the pH of normally neutral pharyngeal secretions is likely an irritant to pharyngeal, laryngeal, and tracheal mucosa. This inflammatory condition, in an already traumatized or intubated airway, may well be the nidus for development of LTS.

The implications of GERD and LTS are numerous. It is likely that there is a strong relationship between the development of acquired LTS and the presence of GERD. One could speculate that those patients who have "silent" GERD and subsequently have a traumatized airway for whatever reason would be good candidates for the development of acquired LTS. It is known that many patients who develop a traumatized airway from intubation or other injury will heal without development of LTS. The presence or absence of "silent" GERD may be the key to a patient's developing or avoiding acquired LTS. One may also ask which comes first. It is likely that some patients have GERD prior to laryngeal trauma and that this is a factor in the production of the stenosis. On the other hand, trauma or manipulation of the airway may induce the patient to have reflux and would be, again, a factor in the production of the stenosis. It is thought that there is sufficient information to suggest that treatment of GERD is essential in the overall management of LTS.

The relationship between idiopathic SGS and pharyngeal acid reflux events in female patients has been previously noted.[5,6] These patients likely behave somewhat differently, in that they probably have significant GERD before they develop SGS. The predominance of this type of stenosis in women has yet to be fully explained.

The timing of 24-hour pH monitoring is difficult. During the critical stage of their illness, patients cannot undergo an esophagram, let alone pH testing. In this study, these procedures were done as soon as the patient was medically stable. It is felt that these results would have been more remarkable if studies had been done during the critical portion of the development of LTS. Antireflux treatment was instituted on first contact with the patient,[10] and when the patient was ready for study, was withdrawn 3 days prior. The effect of early antireflux treatment may also have affected the outcome of the pH study. About 20% of normal controls will have pharyngeal acid reflux events. In this group, injury is likely avoided because these normals have excellent protective mechanisms to prevent the effect of reflux. In this study, 4 of 12 LTS patients did not exhibit pharyngeal acid reflux events, possibly due to the variability of reflux events; it is possible that pharyngeal acid reflux events did not occur during the 24-hour study duration, or that reflux did not play a role in the pathogenesis of their LTS.

Smit et al[13] have reported an innovative 2-site method of study in which the upper probe is endoscopically placed in the upper esophageal sphincter; the method does not require manometric study prior to probe placement. This method is simple and may give as much information as the 3-site method. A comparative study of these 2 methods is planned.[13]

This study supports the original conclusions of Koufman.[1] The majority of LTS patients have pharyngeal acid reflux events. This relationship is more than casual, and all patients with LTS should have treatment of GERD along with management of the stenotic lesion. On a long-term basis, identification of the presence and extent of GERD is necessary to prevent further supraesophageal complications.

CONCLUSIONS

1. The critically ill patient with a traumatized or intubated airway is at high risk of developing LTS if there is significant GERD.

2. It is suggested that vigorous antireflux management be instituted on initial contact with the critically ill patient with a traumatized or intubated airway.

3. Pharyngeal acid reflux events are significant in LTS patients, as measured by 3-site 24-hour pH monitoring.

4. Identification and treatment of GERD in patients will significantly simplify and improve treatment for LTS.

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THIRTY-EIGHT–YEAR EVALUATION OF A SURGICAL TECHNIQUE TO PROTECT THE EXTERNAL BRANCH OF THE SUPERIOR LARYNGEAL NERVE DURING THYROIDECTOMY

JOHN M. LORÉ, JR, MD
STOYAN I. KOKOCHAROV, MD
SOL KAUFMAN, PHD
ALLEN RICHMOND, PHD
NAN SUNDQUIST, RN
BUFFALO, NEW YORK

The purpose of this study is to evaluate a surgical technique for the preservation of the function of the external branch of the superior laryngeal nerve (EBSLN) during total thyroid lobectomy and total thyroidectomy. Permanent injury to this nerve can be a disaster, especially in singers and professional speakers who depend on control of pitch, and a clear and forceful voice. Voice changes may be either obvious or subtle. For better voice analysis, a detailed questionnaire is necessary. Thus, the evaluation in this study is based on laryngoscopy of 934 nerves in 675 patients and detailed subjective voice evaluation of 66 patients during the last 2 years of the study. There are 2 surgical principles. First, the EBSLN is not routinely exposed; the distal 1.5 to 2.0 cm (critical area) of the superior thyroid vessels are carefully dissected, exposed, and ligated, preferably independently. Careful observation ensures that the EBSLN is not included in the ligature. Second, the ultimate evaluation of the surgical technique is the effect of voice changes on the patient's lifestyle. Laryngoscopy of 934 nerves found bowing, temporary in 4 patients and permanent in 1 patient (limited follow-up). Of the 66 patients with voice evaluations, 14 had changes: 9 had temporary changes, while 5 had permanent changes. In these 14 patients, voice changes had no effect on lifestyle in 13, and the effects were indeterminate in 1 (unavailable for follow-up). The estimated deleterious effect of voice changes on lifestyle is no greater than 1.5% of the 66 patient responders.

KEY WORDS — external branch superior laryngeal nerve, questionnaire, thyroidectomy, voice changes.

INTRODUCTION

This study was undertaken to present and evaluate a surgical technique for thyroidectomy that would minimize any permanent effect of voice changes on a patient's lifestyle related to the external branch of the superior laryngeal nerve (EBSLN). The rationale is to compare the technique with others in the literature and compare our results based on laryngoscopy and a voice questionnaire.

MATERIAL AND METHODS

This report is relative to a 38-year experience (December 1958 to July 1996) evaluating 934 EBSLNs in 675 patients, which were placed at risk during thyroidectomy. Four hundred total thyroid lobectomies and 267 total thyroidectomies were performed. Not included in this study were 10 patients who had subtotal thyroidectomies (rarely performed) and 12 patients who had previous surgery done elsewhere. Temporary and permanent paralyses were evaluated. All of these patients were operated on by the same surgeon (J.M.L.). This is a nonrandomized study. The surgical technique used in the entire period was basically the same, with minor variations. The evaluation of results is based on laryngoscopy on all patients (phase 1 of the study was prospective) and a detailed subjective voice evaluation of 66 patients during the last 2 years (phase 2 of the study was retrospective).

The laryngoscopy phase included all 934 nerves placed in jeopardy during the entire 38-year period. This study is primarily based on preoperative and postoperative laryngoscopy, with mirror early in the series and almost exclusively with optical instruments, flexible and rigid, later in the series. Immediate postoperative direct laryngoscopy (not optical) has no place in the evaluation of vocal cord function. The primary abnormal finding was considered to be the presence of bowing of the vocal cord. Inferior displacement of the vocal cord does not appear to be as easily detectable.

During this study, more detailed evaluation of voice problems became indicated, and it was obvious that the laryngoscopy could not detect fine tuning of the vocal cords. This observation is supported by others.[1-3] The subjective voice evaluation of the 66 patients who responded out of 102 questionnaires sent was the basis of detailed evaluation of the voice. In addition, more data were obtained from 3 nonprofessional singers in this latter group who gave the

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From the John M. Loré, Jr, MD Head and Neck Center at Sisters of Charity Hospital, Buffalo, New York.

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CORRESPONDENCE — John M. Loré, Jr, MD, Head and Neck Center, Sisters Hospital, 2157 Main St, Buffalo, NY 14214.

same information, namely, high pitch range changes. The use of videolaryngoscopy and videostroboscopy was very limited, since this phase of the study was retrospective.

The early patients (111 nerves) did not receive steroids; the later patients (823 nerves) received steroids preoperatively, intraoperatively, and postoperatively to reduce edema and temporary nerve weakness. Steroids were used mainly to prevent bilateral recurrent laryngeal nerve (RLN) paralysis. The statistical analyses are based on χ^2 testing for difference in incidence rate.

Anatomy. The EBSLN arises from the ganglion nodosa and supplies the cricothyroid (CT) muscle, as well as the pharyngeal plexus and the inferior pharyngeal constrictor muscle. It also has a communication with the superior cardiac nerve via a branch of the sympathetic chain.[4,5] The EBSLN is closely related to the superior thyroid artery and veins and usually passes medial to these vessels and then either lies anterior to the inferior pharyngeal constrictor muscle or within it to reach the CT muscle. Or, it can proceed with the vessels and leave them at various distances from the thyroid capsule to reach the CT muscle. One explanation of the contracture of the CT muscle tilts the thyroid cartilage forward and the cricoid cartilage backward. Another explanation states that contracture of the muscle tilts the anterior part of the cricoid cartilage cranially and carries the arytenoid cartilage dorsally.[4] In any event, the vocal cord is lengthened (stretched) to produce tensing of the vocal cord that is responsible for high pitch and other voice changes.[6]

There are 2 parts to the CT muscle: the anterior-superior and the posterior-inferior. Branching of the EBSLN has been seen just before the nerve enters the 2 portions of the CT muscle. Hence, these branches to the 2 parts of the CT muscle are most often distal to the EBSLN relationship to the superior thyroid vessels. Two to 4 branches have been described by Kambic et al[7] before entry into the CT muscle or within the muscle. There is evidence that the EBSLN via connection with the RLN has been shown to reach the vocalis muscle, a portion of the thyroarytenoid muscle that is also supplied by the RLN.[8,9] This may well influence the tension of the free edge of the vocal cord and possibly add another parameter to evaluation of function of the EBSLN and the RLN relative to voice change.[4] The superior pole vessels enter the thyroid capsule inferior to the most superior portion of the superior pole of the thyroid. That is, they are not located at the top of the superior pole.[10]

Surgical Technique. The high points of the surgical technique are as follows.[11,12] 1) Superior pole exposure is the last step in thyroidectomy. 2) The artery and veins are exposed 1.5 to 2.0 cm proximal to their penetration of the thyroid capsule. 3) Vessels are ligated and divided as close to the thyroid capsule as feasible without a clamp on the proximal stump. 4) Ideally, the artery and the veins are separately dissected and ligated. The artery and veins are ligated together when small veins are adherent to the artery or its branches, after careful evaluation ensures that the nerve is not entrapped.

The superior pole dissection and ligation of the superior thyroid artery and veins are performed as a final step in thyroidectomy. The purpose of this ligation as the last step in thyroidectomy is to facilitate the best exposure possible for dissection of the superior pole vessels for at least a distance of 1.5 to 2.0 cm (critical area) from their entry into the thyroid capsule to avoid injury to the EBSLN, which may be closely related to the vessels in this area. Nevertheless, many surgeons prefer initial ligation of the superior pole vessels at the onset of the operation. To fully mobilize the thyroid lobe, the anterior and posterior[13,14] suspensory ligaments of the thyroid must be transected. In total thyroid lobectomy, the isthmus is transected along the contralateral side. In total thyroidectomy, the isthmus may or may not be transected, but is at least partially mobilized along with the transection of the ipsilateral anterior and posterior suspensory ligaments. At this point, the lobe is virtually hanging (Fig 1[11,12]) from the superior pole attachment, unless the superior pole extends superiorly to the level of the superior edge of the thyroid cartilage or the thyrohyoid membrane. Under these latter circumstances, additional exposure is necessary by further mobilization of the superior skin flap, extending the original thyroid skin incision laterally. Transection of the strap muscles may also be necessary if the gland is significantly enlarged.[15] Repair of these muscles may be important relative to voice quality.[16] Very rarely will a separate small horizontal skin incision be required at the level of the thyrohyoid membrane for adequate exposure of the highly placed superior pole. In the area of the thyrohyoid membrane, the internal branch of the superior laryngeal nerve may be placed at risk as the dissection proceeds inferiorly.[17] The EBSLN may be closely related to the superior thyroid vessels, usually medial. To improve exposure, gentle downward and slightly lateral traction (at times, medial traction) is applied to the thyroid lobe. During this maneuver, care must be taken to avoid stretching the nerve.[18,19] At the same time, exposure of the "critical" area is done by gently displacing superiorly the soft tissue surrounding the vessels, starting at the superior pole

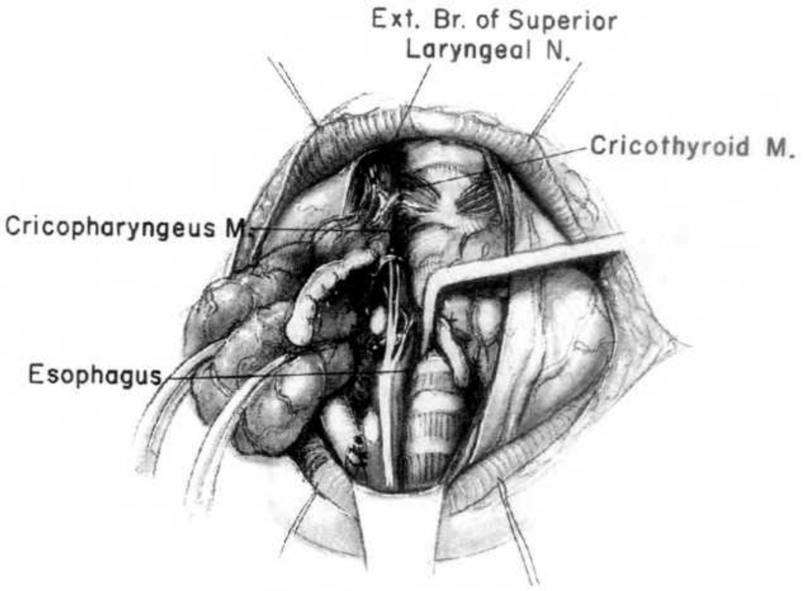

Fig 1. Right thyroid lobe is completely mobilized, leaving its attachment at superior pole. With gentle downward and slight lateral traction, superior pole vessels have been cleared for distance of 1.5 to 2.0 cm (critical area). External branch of superior laryngeal nerve is seen entwined and hooked by vessels before it reaches cricothyroid muscle. Further inferiorly, recurrent laryngeal nerve sends 1 branch to esophagus and another superiorly and laterally, divides, and passes deep to posterior suspensory ligament. (Reprinted with permission.[11,12])

of the thyroid. This enhanced exposure appears to be more difficult if the ligation of the superior pole vessels was done as the initial step in thyroidectomy. Ideally, and most often, the artery and vein are dissected and separated from one another. The vessels are independently ligated as close to the thyroid capsule as possible.[11,12] This aids in the protection of the nerve, if in fact the nerve is located in this critical area. This site of ligation is below the tip of the superior pole of the thyroid gland. Clamps are never used on the vessels proximally. The artery is usually a single structure; its branches usually occur just before the vessels enter the gland below the top of the superior pole of the thyroid. One should avoid any mass ligature of the superior pole of the thyroid with the vessels,[10] since thyroid tissue will remain and injury to the nerve may occur. The venous drainage may be different, in that rather than 1 main artery, there may be more small veins within the vascular bundle. Occasionally, there are significant adhesions between the artery and small veins. Separation of these vessels may cause bleeding, which may be very difficult to control without injury to the nerve. When the vessels are adherent to one another, they are encompassed in a single ligature (3-0 or 2-0 silk) with a distal suture ligature (4-0 silk, neurosurgical needle). These adhesions may occur in Hashimoto's thyroiditis, Graves' disease, and carcinoma. If the EBSLN is encased in carcinoma and there was preoperative bowing, then sacrifice of the nerve is of little concern. During this dissection, the EBSLN may be "hooked" and looped[11,12,20] around a branch of the artery, and would thus be very vulnerable to injury (Fig 2[11,12]).

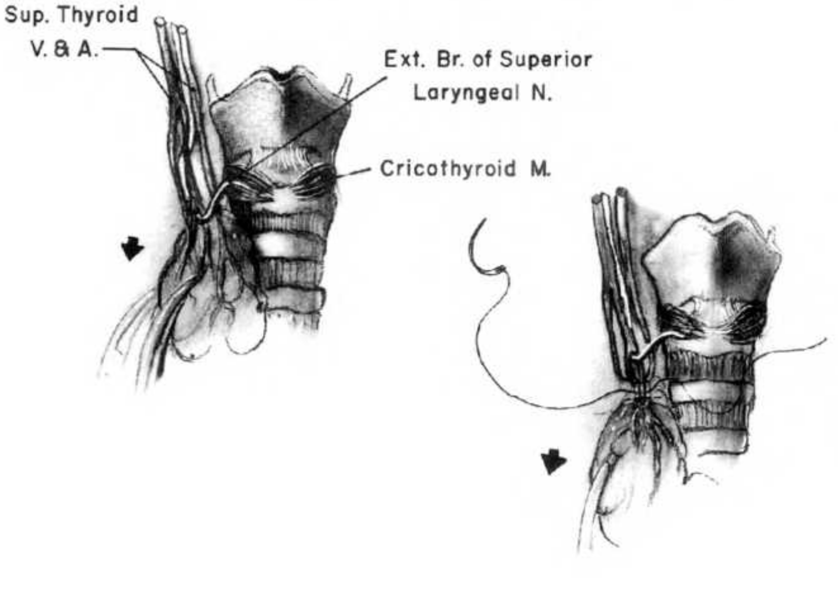

Fig 2. Vessels are visualized and separated. External branch of superior laryngeal nerve is seen hooked around branch of artery in critical area. Vessels (vein and artery; V. & A.) and nerve are now cleared of overlying soft tissue to show how nerve can be related to vessels. Vessels do not enter tip of superior pole, but somewhat inferior. Sutures are placed as close as possible to capsule of thyroid. Dotted line indicates site of transection; arrows indicate direction of gentle downward traction. (Reprinted with permission.[11,12])

TABLE 1. RESULTS ON VOICE CHANGES QUESTIONNAIRE IN 66 PATIENTS (96 NERVES)

Voice Changes	*Temporary*	*Permanent*
High pitch	3	3
Hoarseness	3	1
Fatigability	3	1
14 Patients (24 nerves; 21%)	9 (13.6%)	5 (7.6%)

TABLE 2. RESULTS ON LIFESTYLE QUESTIONNAIRE IN 66 PATIENTS (96 NERVES)

No effect on lifestyle in 65 patients

Other patient not available for follow-up

Estimated deleterious effect of voice changes on lifestyle is no greater than 1.5%

Careful observation of the nerve is done. Identification of the nerve with this technique occurs approximately 10% of the time. Verification can be done with a nerve stimulator at 0.5 mA. The CT muscle will be seen to contract. One should avoid overstimulation of the nerve. No further search is done for the nerve, since all the adherent tissue surrounding the vessels in the critical area has been freed. With the careful and meticulous dissection of the superior pole vessels, further search for the nerve not only is unnecessary, but can be detrimental.[2] Electrodissection or coagulation is totally contraindicated in this region[2] and is not recommended deep to the superficial cervical fascia except for minimal bleeding from the thyroid gland. Cottonoid strips are utilized in the vicinity of the nerve rather than standard gauze. Prior to closure, the wound is filled with saline and observed for any bleeding. Bleeding is checked by a Valsalva maneuver up to 40 to 50 mm H2O. Lightening the anesthesia to induce bucking may serve the same purpose. A small Jackson-Pratt drain is used. This latter technique is controversial.

RESULTS

The results are evaluated based on prospective laryngoscopy of 934 nerves at risk in 675 patients and then retrospective voice evaluation of 66 patients (respondents of 102 patients who were sent questionnaires) with 96 nerves at risk during the last 2 years of the study.

There were 4 (0.4%) bowed vocal cords with temporary paralysis and 1 cord (0.1%) with permanent paralysis of the EBSLN evaluated by laryngoscopy. The 1 patient whose paralysis was "permanent" had limited follow-up; her voice improved, but minimum bowing persisted. An interesting statement by this patient was that she was "not singing as loud" immediately after surgery; but at the last follow-up at 36 weeks, prior to the patient's cardiac death, she stated "I can yell like crazy." During this period of time, the bowing, which was minimal, had not improved. The temporary bowing of the 4 other patients lasted 2.5, 4, 4, and 18 weeks, respectively. After initial review of these data, it became obvious that the question "How is your voice?" was inadequate. Hence the detailed voice questionnaire, as well as specific queries by telephone and/or repeat follow-up with patient visits as indicated. The results are in Table 1. Finally, additional data regarding voice changes relative to effect on lifestyle are reported in Table 2. Table 1 depicts the results of a detailed and condensed questionnaire (1 page) mailed to 102 patients who were operated on during the last 2 years of the study. Sixty-six patients (96 nerves) responded. In 14 patients (24 nerves) with voice changes, the EBSLN was not identified, while in the remaining 52 patients (72 nerves) without voice changes, 8 EBSLNs were identified. Statistically, the difference between these 2 groups relative to the nerve's being exposed or not exposed is not significant ($p = .11$).

Although the EBSLN was not identified in any of the 14 patients with voice changes, 1 patient (in the temporary group) had bowing of the left vocal cord with a temporary RLN paralysis of the right vocal cord. The part played by the vocalis muscle is impossible to state. This laryngoscopy finding of bowing was the only one in 14 patients with voice changes (incidence of 7.1%). The effect of voice changes on lifestyle appears to be a very important clinical evaluation as far as the patient is concerned and is the ultimate evaluation of the surgical technique. In the temporary voice change group, 3 patients were nonprofessional singers with high pitch dysfunction. After 24 months, 1 of these patients stated that she was now "singing [her] usual part in the chorus," and another patient, now 30 months postoperative, states that his difficulty holding "E above middle C" has abated following weight lifting and isometric and isotonic exercises to strengthen neck and shoulder muscles. The strength and pitch control of his voice is normal. In addition, when he plays his saxophone he no longer has quivering as he blows into the mouthpiece. Extralaryngeal skeleton effects may be more significant than realized.[16] The third patient states that her pitch is low but she had intermittent hoarseness preoperatively. She states that she has dropped out of the choir but is "not depressed." She is a realtor and has voice fatigue late in the day, but she states this has not affected her lifestyle. Subsequent videostroboscopy revealed a small vocal cord nodule.

In 675 patients (934 nerves), steroids were used in 575 patients (823 nerves). Three of these patients (0.4%) had temporary, but no permanent, bowing on laryngoscopy, lasting 2.5, 4, and 18 weeks. No steroids were used in the other 100 patients (111 nerves); 1 (0.9%) had permanent bowing, and 1 (0.9%) had bowing lasting 4 weeks.

DISCUSSION

The diagnosis of EBSLN paralysis can very easily be made when there is gross abnormality of the larynx, for example, bowing or, less detectable, downward displacement of a vocal cord. Laryngoscopy is a relatively easy method as far as bowing is concerned. However, this is only part of the diagnostic dilemma, since the other concern is primarily voice changes. Laryngoscopy can be supplemented by videolaryngoscopy and videostroboscopy, while the evaluation of the voice changes extends from patient evaluation to various tests that include electroglottography (EGG), flow glottography (FGG), and Visi-Pitch. These tests were used very minimally, since this portion of the study was retrospective. Although we have no personal experience with electromyographic evaluation, its pitfalls include the exact location of the electrode.[21] Complicating the evaluation and diagnosis is the fact that findings on laryngoscopy and even videostroboscopy can be relatively normal despite voice changes. To indict the EBSLN, all other causes of voice changes must be eliminated, for example, RLN abnormalities, endotracheal intubations (eg, mucosal lesions, arytenoid dislocations),[22] and transected muscles, as well as any local mucosal disease of the vocal cords, including that caused by hypothyroidism or aging. The cause of a slightly wavy,[23] irregular vocal cord mucous membrane may be difficult to ascertain. This may be due in part to the vocalis muscle or the CT muscle. This becomes difficult to evaluate, since the vocalis muscle may have a motor supply from either the RLN or the EBSLN or a combination of both.[8,9] It appears that when only voice changes are present, the diagnosis of EBSLN injury may be on an exclusion basis alone. One voice change, in particular — the loss of high pitch tone in a singer's voice — appears to indict the CT muscle, but there still is the question of the influence of the vocalis muscle. The end point of voice change, regardless of the various tests, is the patient's complaints and, most important, whether the voice change has an effect on the patient's profession and/or lifestyle. Other voice changes can usually be handled by adaptation of the patient, except for the singer. Since adaptation is not possible with all voice changes, the question is how a surgeon can best perform thyroidectomy without compromising the patient's lifestyle.

An important step in thyroidectomy is the handling of the superior pole of the thyroid gland and the site of ligation of the superior pole vessels. This must be done with the utmost care and precaution. The technique may vary, but the EBSLN must not be injured with the ligature or by clamping the vessels indiscriminantly. One of the safer ways is to place the ligature as close to the thyroid capsule as possible after "clearing" of the vessels in the "critical area." If possible, the separate ligation of the vessels is recommended by most surgeons and supported by this study. The vessels, especially the smaller veins, at times could be injured, and they are best left exposed but not completely dissected. Once vessels are carefully exposed and the critical area is cleared, it is mandatory to avoid injury to an inferiorly located EBSLN. It does not appear necessary to actually expose the nerve to avoid it. The less dissection to locate the nerve, the better, yet some authors seem to have virtually the same success rate by attempting to locate the nerve, as noted below.

Approximately 20% of nerves pass into the inferior pharyngeal constrictor muscle prior to the termination in the CT muscle, and they should not be exposed, since bleeding can occur.[2] Moosman and DeWeese[18] found 11% of the nerves to pass through the lower fibers of the inferior pharyngeal constrictor muscle, compared with 15% in the series of Durham and Harrison.[24]

Kark et al,[1] who strongly recommend attempting to expose the EBSLN in all situations, were not able to identify the nerve in 16% of the operations. They had very good results. Lennquist et al,[2] who selectively expose the nerve, did not do so in 28% of the operations and also had very good results. Cernea et al[25] attempt to locate the nerves in all patients utilizing the nerve stimulator, but were unable to expose the nerve in 2 patients of 28. Despite the last 3 groups' abilities, not all the nerves were able to be identified. Lekacos et al,[26] who had the best results (Table 3[1-3,18,25-32]), preserved nerve function in all of 122 patients by individual ligation of vessels. This is difficult to understand when in Kark et al's control group of 100 patients (following radical mastectomy with endotracheal anesthesia), 3 patients had permanent (1 severe) voice changes. This was ascribed to the endotracheal anesthesia and not to any thyroid surgery.[1] The results of our study relative to EBSLN exposure are compared with those of other authors on Table 3.

In the data of Kark et al[1] (who recommend identification of the EBSLN in all patients) reporting 38 patients with 56 nerves at risk, 47 nerves were found and preserved (84%). These are very similar to the data of Lennquist et al[2] (they selectively identify the nerve), who report that 36 of 50 nerves could be identified and 72% were preserved. Thus, the nerve was

TABLE 3. NECESSITY AND RELIABILITY OF EBSLN EXPOSURE FOR ITS PRESERVATION

Authors	*Year*	*Surgical Technique Recommended*	*Evaluation Methods*	*Results*	*p*
Teitelbaum and Wenig[3]	1995	Separate ligation of superior pole vessels; identifying nerve is not routine	Videostroboscopy, EMG, interview	Total 20 patients (26 nerves); 1 patient (5%) with nerve injury	.68 (NS)
Cernea et al[25]	1992	Identify nerve with nerve stimulator near superior thyroid pole	Laryngoscopy, phonoaudiology, EMG	Total 26 patients; no patients with nerve injury; in 24 patients (92%), nerve was identified	.14 (NS)
Lennquist et al[2]	1987	Visualize EBSLN whenever possible without intramuscular dissection	Laryngoscopy, questioning	Total 38 patients (50 nerves); 1 patient (2.6%) with permanent voice changes; 36 (72%) nerves were identified	.29 (NS)
Lekacos et al[26]	1987	Separate ligation of superior pole vessels; no exposure of nerve	Laryngoscopy; high pitch?	Total 122 patients (227 nerves); none with nerve injury	.002 (SS)
Friedman and Toriumi[27]	1986	Identify nerve			N/A
Kark et al[1]	1984	Identify and preserve EBSLN	Questionnaire, oscilloscopy, laryngoscopy	Total 38 patients (56 nerves); 2 patients (5%) with permanent voice changes; 47 (84%) nerves were identified	.64 (NS)
Kark et al[1] (control)	1984		Questionnaire,* oscilloscopy, laryngoscopy	Total 100 patients; 3 patients (3%) with permanent voice changes	.17 (NS)
Jacobs et al[28]	1983	Avoid mass ligation of superior thyroid artery and vein			N/A
Droulias et al[29]	1976	Identify nerve before ligation			N/A
Thompson et al[30]	1973	Clamp vessels separately; not necessary to identify nerve			N/A
Reeve et al[31]	1969	Expose nerve to prevent damage	Laryngoscopy, voice assessment	Total 100 patients (157 nerves); 3 patients (3%) with voice changes; 92 (48%) nerves were identified	.17 (NS)
Moosman and DeWeese[18]	1968	Isolate and clear vessels with individual ligation			N/A
Moran and Castro[32]	1951	Dissect and ligate superior thyroid artery			N/A
This study		Not necessary to expose nerve	Laryngoscopy, questionnaire, interview	Total 66 patients (96 nerves); 5 patients (7.5%) with permanent voice changes; 8 (33%) of 24 nerves were identified	χ^2 Test results above

p Values are historical control compared to this study.

EBSLN — external branch of superior laryngeal nerve, EMG — electromyography, NS — not significant, SS — statistically significant, N/A — not applicable.

*Voice assessments on 100 women 6 months after endotracheal intubation.

not located in 16% in the Kark et al series or in 28% in the Lennquist et al series. In the Kark et al series, there were 9 patients with voice changes: 7 temporary and 2 permanent, for a 5% incidence in patients with permanent voice changes. Our data (with the nerve not routinely identified nor specifically searched for) reveal 5 patients with permanent voice changes (1 patient with limited follow-up) of the 66 patients who responded to the 102 patient questionnaires. This is an incidence of 7.6%, which is not significantly different from the previous experience.[1,2] The p values compared to our study are Kark et al, $p = .64$, and Lennquist et al, $p = .29$. Comparison with the 3% incidence in the Kark et al control group of 100 patients who had radical mastectomy and endotracheal intubation shows no significant difference ($p = .17$). Thus, statistically, the voice changes (3%) seen in thyroidectomy could be due to endotracheal anesthesia. Another point not mentioned by any of the 47 authors we reviewed were the ecological effects. For example, thyroidectomies performed in the New York City area are quite different from those in western New York. In the latter area there is a preponderance of fibrous tissue reaction surrounding the thyroid gland. This is associated with a high incidence of Hashimoto's thyroiditis in western New York. This appears to pose greater risk of nerve injury, whether it be temporary or permanent, and whether it be RLN or EBSLN. This pathologic finding may be related to possible chemicals in the drinking water.

In the analysis regarding surgical technique, it is not quite correct to compare identification of the RLN with that of the EBSLN as some authors suggest. All the RLNs must be exposed within the entire operating field for preservation, but this type of exposure is not necessary to preserve the EBSLN.

CONCLUSIONS

The surgical technique described affords reasonable protection of the EBSLN during thyroidectomy. Routine exposure of the nerve does not appear to be necessary. Individual ligation of vessels is recommended unless there are adhesions of the small vessels to the artery. Ligation of the vessels is performed in an area of 1.5 × 2.0 cm immediately proximal to the thyroid capsule area, which is cleared of soft tissue to protect the EBSLN. Although the function of the EBSLN appears to be normal on mirror or optic laryngoscopy, there are other factors involved in the fine tuning of this nerve that cannot be seen on laryngoscopy. When bowing was evaluated by laryngoscopy in all 934 nerves (675 patients), there was only 1 patient, with limited follow-up, whose bowing was minimal but permanent (0.1%). Of the 66 patients evaluated by our retrospective patient voice questionnaires, 9 had temporary changes and 5 had permanent changes. Of these 14 patients, 13 found no effect on lifestyle, and in 1 the effects were indeterminate (unavailable for follow-up). The estimated deleterious effect of voice changes on lifestyle is no greater than 1.5%, based on 66 patient responses.

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OTOPLASTY AND RECONSTRUCTION OF AURICULAR DEFECTS

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RETICULAR FIBERS IN THE VOCAL FOLD MUCOSA

KIMINORI SATO, MD, PHD
KURUME, JAPAN

Light and electron microscopic investigations of the reticular fibers (RFs) in the vocal fold mucosa were carried out on excised human adult larynges. The results are summarized as follows. Reticular fibers were found in the superficial and intermediate layers of the lamina propria of the vocal fold mucosa. They were most abundantly discovered around the vocal fold edge, and they decreased toward the superior and inferior portions of the vocal folds. The RFs were composed of slender fibrils, about 40 nm in diameter, and having cross-bands with a periodicity of about 67 nm. They were found in close association with the basal lamina of the epithelium and blood vessels. The slender fibrils of the RFs did not form any bundles, but branched and anastomosed. The RFs formed delicate 3-dimensional networks, and the spaces among the fibers were relatively large. Glycoprotein and glycosaminoglycan (proteoglycan) were situated around the RFs and in the spaces among the fibers. Elastic fibers were located in the spaces among the RFs. The 3-dimensional structure of the RFs in the vocal fold mucosa, first demonstrated in this study, appears to be one of the key components of the structural maintenance and viscoelasticity of the vibrating vocal fold tissue.

KEY WORDS — collagenous fiber, extracellular matrix, larynx, reticular fiber, ultrastructure, vocal fold.

INTRODUCTION

The viscoelastic property of the lamina propria of the vocal fold mucosa is very important for the vibratory behavior of the structure. It greatly depends on the extracellular matrix.

Reticular fibers (RFs) are now known to be simply one of several molecular species of collagen.[1] However, it is useful for descriptive purposes to retain the term RF, because their fibrous elements are smaller than, and arranged differently from, the more prevalent collagenous fibers.[1] Our previous investigation revealed that the predominant type of collagen in the lamina propria of the vocal folds is type III.[2] Type III collagen appears to be a major constituent of the slender, 50-nm or less fibers that have traditionally been called RFs.[1]

The purpose of this study was to investigate the 3-dimensional (3-D) structure of RFs, which make up a characteristic collagen and one of the important extracellular matrices in the vocal fold mucosa, by light microscopy (LM) and electron microscopy and elucidate their role.

MATERIALS AND METHODS

We used 5 normal human adult larynges obtained from autopsy cases with ages ranging from 32 to 45 (3 men and 2 women).

For LM studies, the specimens were fixed in 10% formalin. They were dehydrated in graded concentrations of ethanol and embedded in paraffin. Silver stain was employed for identification of RFs.

For scanning electron microscopy (SEM), the specimens were fixed in 2.5% glutaraldehyde at 4°C for 2 hours. Epithelium of the membranous portion of the vocal folds was peeled away under the light microscope. After rinsing with cacodylate buffer solution, a chemical digestion method was used. To observe the 3-D structure of reticular and collagenous fibers, elastic fibers and ground substance of the vocal fold mucosa were chemically digested. The specimens were immersed in 2N sodium hydroxide for 5 to 7 days at room temperature. These specimens were washed in physiological saline solution and postfixed in 2% osmium tetroxide with cacodylate buffer solution at 4°C for 2 hours. This was followed by dehydration in a graded series of concentrations of ethanol, and dried by the critical point drying method. The specimens were then sputter-coated with gold and examined under a Hitachi S-800 scanning electron microscope.

For transmission electron microscopy (TEM), the specimens were fixed in 2.5% glutaraldehyde at 4°C for 2 hours. After rinsing with cacodylate buffer solution, they were postfixed in 2% osmium tetroxide with cacodylate buffer solution at 4°C for 2 hours.

To stain them with ruthenium red to make glycosaminoglycan (proteoglycan) apparent, the specimens were fixed in ruthenium red (1,200 ppm, 0.5 mL) with 3.6% glutaraldehyde (0.5 mL) and 0.2 mol/L

From the Department of Otolaryngology–Head and Neck Surgery, Kurume University, School of Medicine, Kurume, Japan.

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CORRESPONDENCE — Kiminori Sato, MD, PhD, Dept of Otolaryngology–Head and Neck Surgery, Kurume University, School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan.

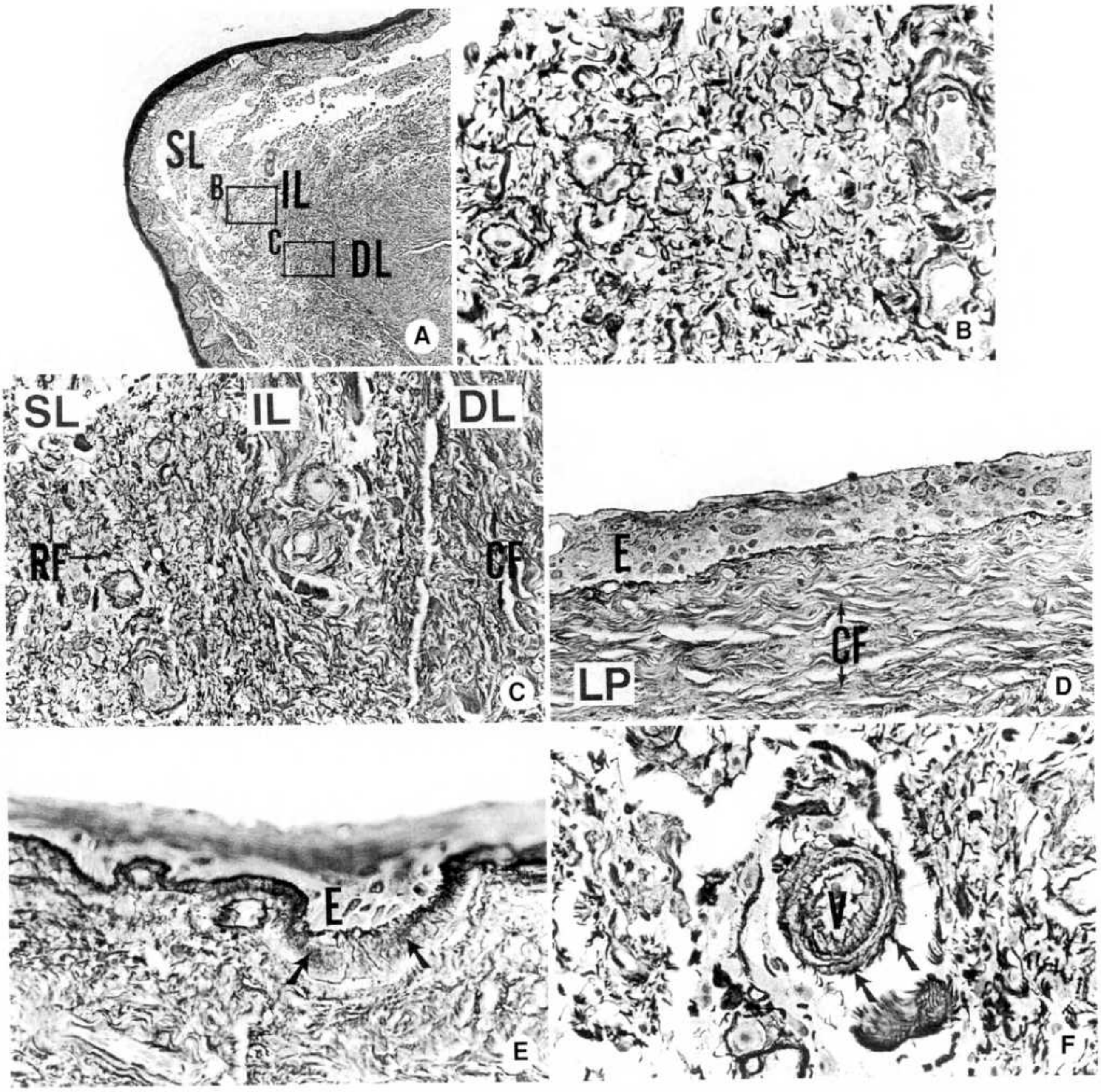

Fig 1. Light microscopic findings in 32-year-old man (silver stain). A) Coronal section of vocal fold (original ×40). SL — superficial layer of lamina propria, IL — intermediate layer, DL — deep layer, B,C — regions magnified in Fig 1B,C. B) Superficial layer of lamina propria of vocal fold (B region on Fig 1A; original ×400). Arrows — reticular fibers. C) Transition areas between superficial layer (SL), intermediate layer (IL), and deep layer (DL) of lamina propria of vocal fold (C region on Fig 1A; original ×200). RF — reticular fibers, CF — collagenous fibers. D) Lamina propria (LP) of inferior portion of vocal fold mucosa (original ×200). CF — collagenous fibers, E — epithelium. E) Reticular fibers (arrows) at basal lamina of vocal fold epithelium (E; original ×400). F) Reticular fibers (arrows) around blood vessel (V; original ×400).

cacodylate buffer solution (pH 7.4, 0.5 mL) at 4°C for 2 hours. After rinsing with cacodylate buffer solution, they were postfixed in ruthenium red (1,200 ppm, 0.5 mL) with 2% osmium tetroxide (0.5 mL) and 0.2 mol/L cacodylate buffer solution (pH 7.4, 0.5 mL) at 4°C for 3 hours.

After rinsing with cacodylate buffer solution, they were then dehydrated in graded concentrations of ethanol and embedded in epoxy resin. Semithin sections were made with an ultramicrotome, stained with 1% toluidine blue, and examined by LM. Thin sections were made with an ultramicrotome and stained with uranyl acetate and lead citrate, tannic acid to make glycoprotein and elastin apparent. The sections were observed under a JEM-2000EX transmission electron microscope.

RESULTS

Distribution of Reticular Fibers in Vocal Fold Mu-

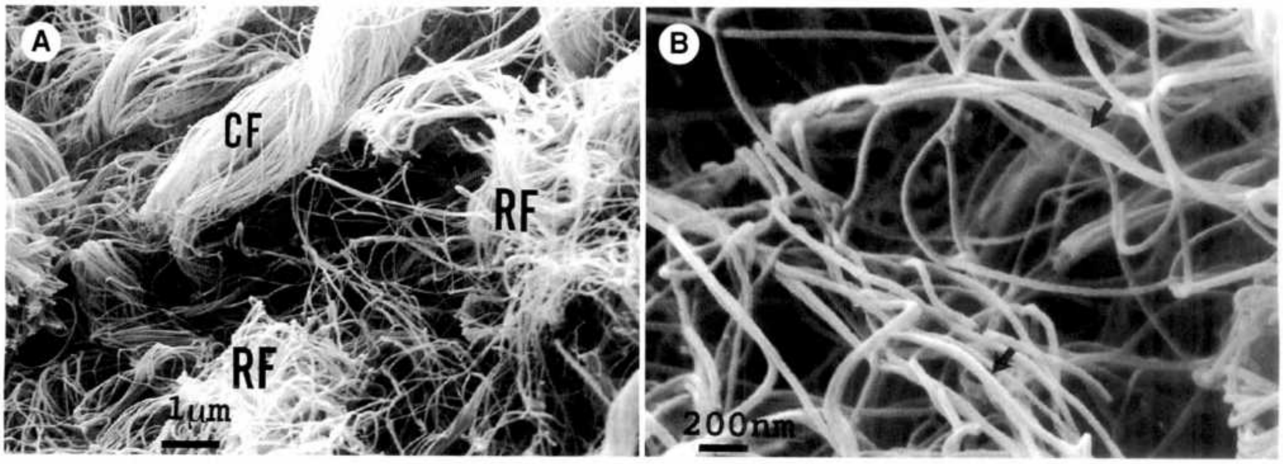

Fig 2. Scanning electron microscopic findings in 45-year-old man. A) Reticular fibers (RF) in superficial layer of vocal fold mucosa. CF — collagenous fibers. B) High magnification of reticular fibers (arrows).

cosa. Figure 1 shows LM findings for the distribution of RFs in the vocal fold mucosa. The RFs, which were thin and stained black with silver stain, were found in the superficial and intermediate layers of the lamina propria of the vocal fold mucosa (Fig 1A, B). They were waving and did not form bundles (Fig 1B). They were most abundantly seen around the edge, and they decreased toward the superior and inferior portions of the vocal folds. They were not detected in the deep layer of the lamina propria of the mucosa (Fig 1C). The RFs were located at the most vibrating portion of the vocal fold mucosa.

Collagenous fibers stained red with silver stain were sparse in the superficial and intermediate layers, and were dense in the deep layer of the lamina propria of the vocal fold mucosa (Fig 1A,C). They formed bundles (Fig 1C). At the superior and inferior portions of the vocal fold mucosa there were no RFs — only collagenous fibers stained red in the lamina propria of the mucosa (Fig 1D). The RFs were not located at the least vibrating portion of the vocal fold mucosa.

Reticular fibers were also found in close association with the basal lamina of the vocal fold epithelium (Fig 1E) and surrounding tissue of the blood vessels (Fig 1F) in the vocal fold mucosa around the edge.

Fine Three-dimensional Structure of Reticular Fibers in Vocal Fold Mucosa. Figure 2 shows SEM findings of the 3-D structure of the RFs in the vocal fold mucosa. Reticular and collagenous fibers alone remained following treatment by the modified sodium hydroxide maceration method. There were many slender fibrils having cross-bands, about 40 nm in diameter, in the superficial layer of the vocal fold mucosa (Fig 2A). The fibrils were slender and branched and anastomosed (Fig 2B). They did not form bundles, but formed delicate 3-D networks (Fig 2). Spaces among the fibers were relatively large (Fig 2B). Some RFs fused with fibrils of collagenous fibers (Fig 2A). Some RFs surrounded bundles of collagenous fibers (Fig 2A).

Figure 3 shows TEM findings of RFs in the vocal fold mucosa. The RFs were made up of unit fibrils (Fig 3A). They were about 40 nm in diameter and had cross-bands with a periodicity of about 67 nm (Fig 3A). The slender fibrils did not form bundles (Fig 3A).

Relatively dense RFs were observed in close association with the basal lamina of the vocal fold epithelium and surrounding tissue of the vessels in the vocal fold mucosa. They were located at the lamina reticularis of the basal lamina (Fig 3B) and the surrounding tissue of the endothelial cells and pericytes of the vessels (Fig 3C).

There were glycoproteins around the RFs (Fig 3D). The fibrils of reticular fibers were covered with glycoprotein, and cross-bands of the RFs could not be seen (Fig 3D). Glycoproteins were distributed around the RFs and in the spaces among the RFs (Fig 3D). There were also glycosaminoglycans (proteoglycans) around the RFs (Fig 3E). The small dots associated with RFs represent granules of proteoglycan (Fig 3E). Proteoglycan granules were attached to the fibrils of the RFs and were connected by ruthenium red–staining filaments (Fig 3E). Glycosaminoglycans (proteoglycans) were situated around the RFs and in the spaces among the RFs (Fig 3E). Elastic fibers were located in the spaces among the RFs (Fig 3F).

DISCUSSION

Adult vocal folds have a layered structure consisting of the epithelium; the superficial, intermediate, and deep layers of the lamina propria; and the vocalis

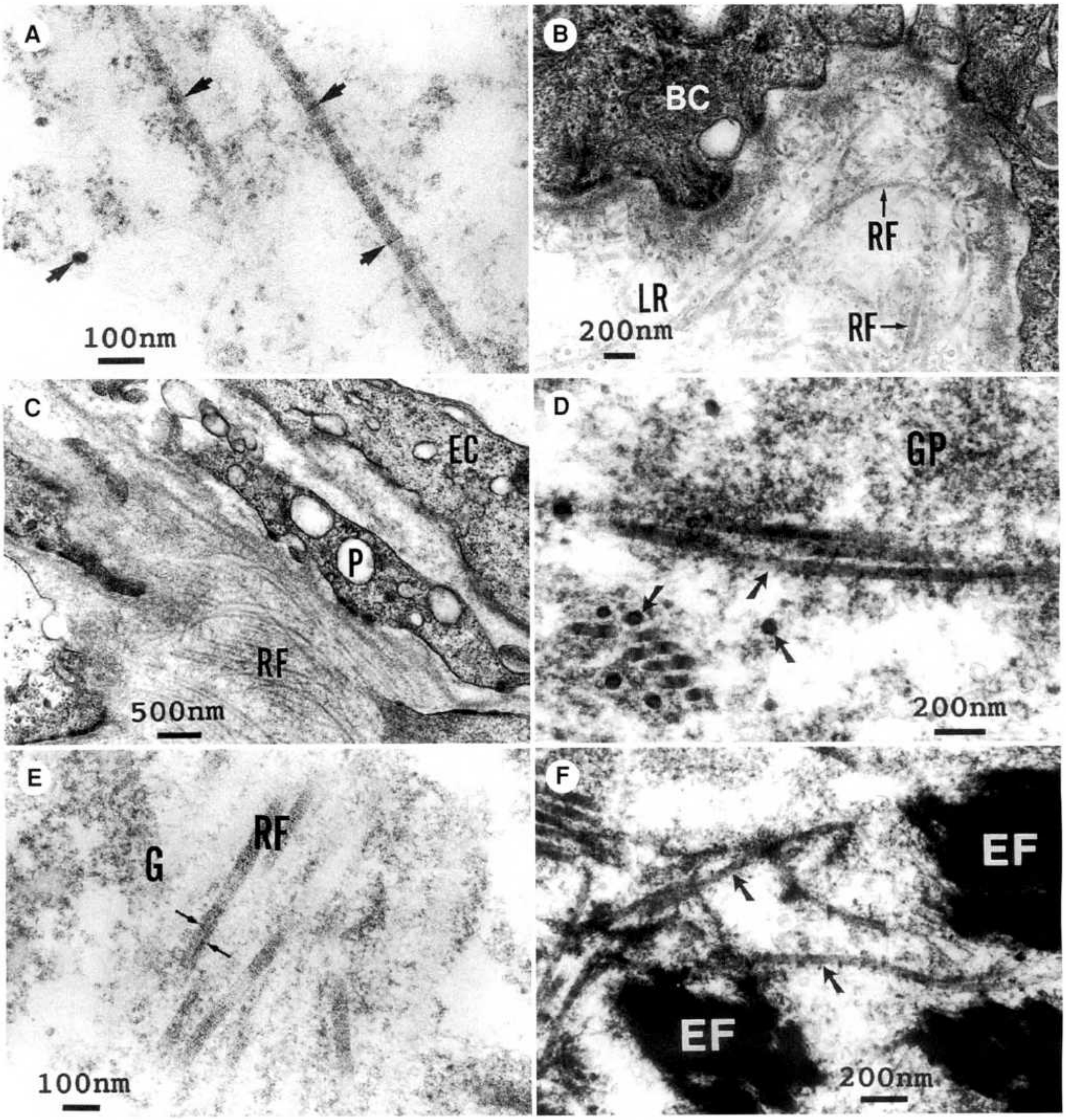

Fig 3. Transmission electron microscopic findings in 34-year-old man. A) Reticular fibers (arrows) in superficial layer of vocal fold mucosa (uranyl acetate and lead citrate stain). B) Reticular fibers (RF) at basal lamina (uranyl acetate and lead citrate stain). BC — basal cell of epithelium, LR — lamina reticularis. C) Reticular fibers (RF) around vessel (tannic acid stain). P — pericyte, EC — endothelial cell. D) Reticular fibers (arrows) and glycoproteins (GP) in superficial layer of vocal fold mucosa (tannic acid stain). E) Reticular fibers (RF) and glycosaminoglycan (G; proteoglycans) in superficial layer of lamina propria (ruthenium red stain). Arrows — granules of proteoglycan. F) Reticular fibers (arrows) and elastic fibers (EF) in superficial layer of vocal fold mucosa (tannic acid stain).

muscle.[3,4] These layers can be divided into 3 sections: a cover, consisting of the epithelium and superficial layer of the lamina propria; a transition zone, consisting of the intermediate and deep layers of the lamina propria or vocal ligament; and a body, consisting of the vocalis muscle.[3,4] The superficial layer is referred to as Reinke's space,[4] and has ground substance, sparse fibroblasts, and sparse elastic and collagenous fibers.[3,4] The intermediate layer is primarily made up of elastic fibers, whereas the deep layer is chiefly composed of collagenous fibers.[3,4] This layered structure is very important in vibration.[3]

It is difficult to explain completely the viscoelastic properties of the lamina propria of the vocal fold mucosa by means of these characteristic distributions of the collagenous and elastic fibers. It is the superficial layer, made up of sparse collagenous and elastic fibers, that vibrates most markedly during phonation. The viscoelastic property of the lamina propria, especially the superficial layer, is very important for the vibratory behavior of the structure. Until now, the fibrous component of the superficial layer of the lamina propria did not receive a great deal of attention. We previously reported that age-related changes in the collagenous and elastic fibers in the superficial layer of the lamina propria contribute to some extent to aging of the voice.[5,6] In this study, the RFs in the lamina propria of vocal fold mucosa were first studied by means of light and electron microscopic investigations.

It is now generally accepted that RFs and collagenous fibers are essentially identical biochemically.[1] The collagenous fibers and RFs have now been found to have different morphologic forms of the same fibrous protein, collagen.[1] The size and pattern of the RFs are different from those of more typical collagenous fibers.[1] It is useful for descriptive purposes to retain the term RF for fibrils composed of collagen 50 nm or less in diameter, and the term collagenous fiber for bundles consisting of unit fibrils 50 to 150 nm in diameter.[1]

Our previous investigation revealed that the predominant type of collagen in the lamina propria of the vocal folds is type III.[2] Type III collagen is one of the fibrillar collagens[7-9] and appears to be a major constituent of the slender 50-nm fibers that have traditionally been called RFs.[1] The collagenous component of RFs is principally type III collagen.[1] In this study, RFs were found in the superficial and intermediate layers of the lamina propria of the vocal fold mucosa. Also, RFs were most abundantly seen around the vocal fold edge. These distributions of the RFs indicate that the RFs are located at the most vibrating portion of the vocal fold mucosa.

Collagenous fibers are flexible, but offer great resistance to any pulling force.[1] Type III collagen fibers (RFs) are thin and result in a more compliant tissue.[9] The function of type III collagen (RFs) is structural maintenance in expansible organs.[1] The RFs in the vocal fold mucosa are composed of slender fibrils, and those fibrils do not form any bundles, but form delicate 3-D networks, and the spaces among the fibers are relatively large. The delicate 3-D structure of the RFs contributes to maintaining the structure of the vocal fold mucosa during vibration without disturbing the vibration.

The supportive functions and viscoelasticity of connective tissue depend largely on the properties of its extracellular matrices. The main extracellular matrices of the vocal fold mucosa (unpublished observations) are RFs, collagenous fibers, elastic fibers, glycoprotein, and glycosaminoglycan (proteoglycan). The fibrils of fibrillar collagen, such as RFs, serve as stabilizing scaffolds in the extracellular matrices.[8] In the vocal fold mucosa, the 3-D structure of the RFs, whose slender fibrils form delicate 3-D networks, possesses innumerable potential spaces. These extracellular interstitial spaces are made up of minute chambers or compartments occupied by other extracellular matrices. In this study, the RFs were covered with glycoprotein and glycosaminoglycan (proteoglycan). Glycoprotein and glycosaminoglycan (proteoglycan) were situated among the spaces of the RFs, and elastic fibers ran through the spaces. One of the roles of glycosaminoglycan (proteoglycan) is to give viscosity to tissues.[7] The main role of the elastic fibers is to give elasticity and resilience to the tissues.[9] The complex of the RFs, elastic fibers, glycoprotein, and glycosaminoglycan (proteoglycan) seems to be very important for the viscoelastic properties of the lamina propria of the vocal fold mucosa, which are very important for the vibratory behavior of a structure.

The RFs were also found in close association with the basal lamina of the vocal fold epithelium and the blood vessels. The most vibrating portion of the vocal folds is the cover, consisting of epithelium and the superficial layer of the lamina propria. The epithelium and blood vessels are attached to the superficial layer via the basal lamina. We previously showed that there are many pericytes around the true capillaries, arterial capillaries, and venous capillaries in the vocal fold mucosa.[10] In addition, the pericytes in the vocal fold mucosa provide mechanical support and protection for the capillary walls, especially during phonation.[10] One of the stressing portions in the lamina propria of the vocal fold mucosa is the junction between the epithelium and lamina propria, and another is that between the basal lamina of the blood vessels and the lamina propria. The delicate 3-D networks of the RFs distributed at these portions may maintain the structure during vibration.

The RFs seem to participate in the pathologic status. Our previous investigation revealed the existence of fibrous long-spacing collagen in the lamina propria of sulcus vocalis.[11] The increased collagenase activity and the presence of RFs are essential to the formation of fibrous long-spacing collagen in vivo.[12] This indicates that the RFs in the vocal fold mucosa are related to viscoelasticity, not only in the physiologic, but also in the pathologic situation.

The 3-D structure of RFs in the vocal fold mucosa appears to be one of the key components of the structural maintenance and viscoelasticity of the vibrating tissue. The complex of RFs and other extracellular matrices seems to be very important for the viscoelastic properties of the vocal fold mucosa.

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EAR SURGERY

The 34th Annual Postgraduate Course in Ear Surgery will be held April 5-10, 1999, in Nijmegen, the Netherlands. For information, contact Prof.dr. P. van den Broek, University Hospital Nijmegen, Department of Otorhinolaryngology, PO Box 9101, 6500 HB Nijmegen, the Netherlands; fax +31 24 3540251; e-mail J.Cornelisse@kno.azn.nl.

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CALCITONIN GENE–RELATED PEPTIDE–LIKE IMMUNOREACTIVE MOTONEURONS INNERVATING THE CANINE INTRINSIC LARYNGEAL MUSCLES

YASUO HISA, MD

NOBUHISA TADAKI, MD
HITOSHI BAMBA, MD

SHINOBU KOIKE, MD
TOSHIYUKI UNO, MD

KYOTO, JAPAN

The percentage of calcitonin gene–related peptide (CGRP)–immunoreactive motoneurons in the nucleus ambiguus innervating the intrinsic laryngeal muscles of colchicine-treated dogs was examined by using cholera toxin B subunit–gold (CTBG) as a retrograde tracer and by immunohistochemistry. Neurons that were labeled with CTBG from the cricothyroid muscle were located in the ventromedial division of the rostral part of the nucleus ambiguus, and the ratio of CGRP-positive neurons was 93.0%. Neurons labeled with CTBG from the thyroarytenoid muscle were located in the dorsal division of the medial part of the nucleus ambiguus, and the percentage of neurons with CGRP was 71.4%. Neurons labeled with CTBG from the posterior cricoarytenoid muscle were located in the ventral division of the medial part of the nucleus ambiguus, and the percentage of CGRP-positive neurons was 85.5%. These findings suggest that the innervation and/or the neurotrophic mechanism involving CGRP for each intrinsic laryngeal muscle is different.

KEY WORDS — calcitonin gene–related peptide, cholera toxin B subunit–gold, intrinsic laryngeal muscle, motoneuron, nucleus ambiguus.

INTRODUCTION

The laryngeal motor innervation system is complex, and the mechanism of innervation of the intrinsic laryngeal muscles remains to be clarified. Possible participation of calcitonin gene–related peptide (CGRP) in motor innervation of the larynx has already been demonstrated.[1] Since the presence of CGRP-like immunoreactive motoneurons (CGRP MNs) in the nucleus ambiguus (NA) was first confirmed by Rosenfeld et al,[2] several investigators have demonstrated that not all motoneurons in the NA are positive for CGRP immunoreactivity.[3-6] We previously reported the interesting fact that there is a distinct difference in the percentage of CGRP MNs in the NA between the canine thyropharyngeal and cricopharyngeal muscles.[7]

Under these circumstances, it seems worthwhile to determine whether there is a difference in the percentage of CGRP MNs in the NA between various intrinsic laryngeal muscles, in order to improve our understanding of the properties of innervation of individual muscles. In the present study, we determined the percentage of CGRP MNs in the NA innervating the canine cricothyroid (CT) muscle, thyroarytenoid (TA) muscle, and posterior cricoarytenoid (PCA) muscle, which differ in function for phonation versus respiration, by using a recently introduced retrograde tracer: cholera toxin B subunit–gold (CTBG).[8]

MATERIALS AND METHODS

The procedure for preparing CTBG has been reported elsewhere.[6] Thirteen puppies of both sexes, weighing 1.5 to 2 kg, were used in this experiment, and all surgical procedures were performed under deep anesthesia with pentobarbital (intraperitoneal administration, 30 mg/kg). The skin was incised on the ventral midline of the neck. The strap muscles were retracted and the larynx was exposed. Five microliters of CTBG solution was injected into several parts of the right CT muscle of 3 dogs, the right TA muscle of 5 dogs, and the right PCA muscle of 5 dogs. After the application of CTBG solution, the injected muscles and surrounding tissues were washed thoroughly with saline, and the skin was sutured.

After a 48-hour postoperative period, 300 μg of colchicine was injected into the lateral ventricle after stereotaxic identification under the same anesthesia described above. Twenty-four hours later, the dogs were perfused through the left cardiac ventricle with 0.1 mol/L phosphate buffered saline (PBS; pH

From the Department of Otolaryngology, Kyoto Prefectural University of Medicine, Kyoto, Japan. This study was supported by a Grant-in-Aid for Scientific Research (No. 03670816) from The Ministry of Education, Science and Culture, Japan.

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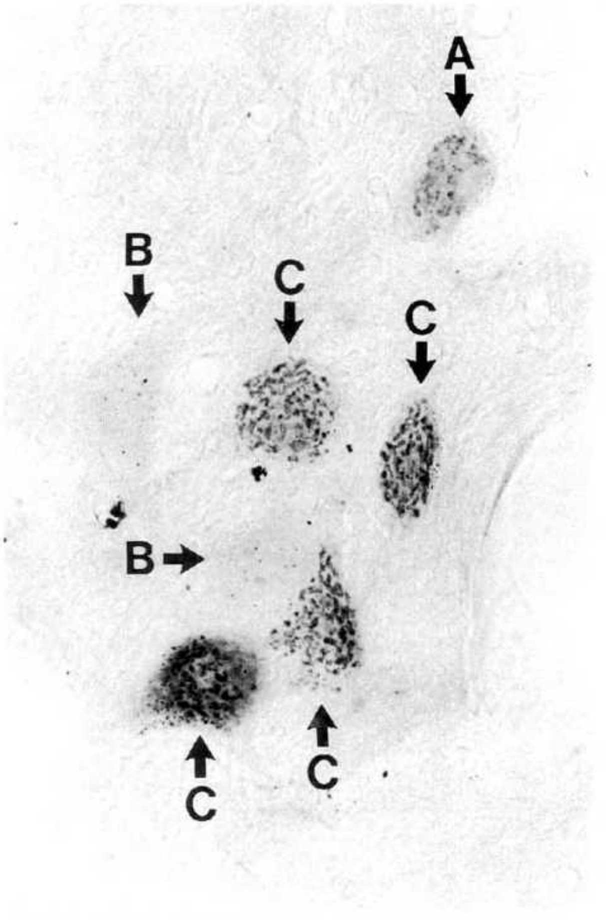
CORRESPONDENCE — Yasuo Hisa, MD, Dept of Otolaryngology, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kyoto 602-8566, Japan.



Motoneurons in nucleus ambiguus after injection of cholera toxin B subunit–gold (CTBG) into posterior cricoarytenoid muscle (original ×340). Motoneurons with only calcitonin gene–related peptide (CGRP) immunoreactivity were identified by presence of brown 3,3' diamidinobenzidine (DAB) products in their cytoplasm (A). Black grains resulting from silver intensification of CTBG were clearly distinguishable from DAB products obtained by immunohistochemistry. Thus, CTBG-labeled motoneurons without CGRP immunoreactivity (B) and CTBG-labeled motoneurons with CGRP immunoreactivity (C) were clearly differentiated.

7.4) followed by an ice-cold fixative containing 4% paraformaldehyde, 0.2% picric acid, and 0.35% glutaraldehyde in 0.1 mol/L phosphate buffer (PB; pH 7.4). After perfusion fixation, the brain stem was removed and postfixed with 4% paraformaldehyde and 0.2% picric acid in 0.1 mol/L PB for 1 day. The extirpated brain stem was then immersed in 0.1 mol/L PBS containing 20% sucrose for 1 day and sectioned at 40-μm thickness with a freezing microtome.

Sections were collected in the 0.1 mol/L PB solution and washed with this solution for 30 minutes at room temperature. Gold labeling was converted to silver grains, by means of a BioCell Silver Enhancing Kit (British BioCell, Cardiff, Wales). After silver intensification, these sections were processed for immunohistochemistry. Free-floating sections were treated with 1% bovine serum for 1 hour and incubated with anti-CGRP serum (Amersham, ×5,000) for 4 days at 4°C. Sections were then incubated with biotinylated anti-rabbit IgG (Vector Lab, ×1,000) for 1 day and with avidin-biotin peroxidase complex (Vector Lab, ×1,000) for 1 day. Sections were washed in 0.1 mol/L PBS. This was followed by incubation with 0.01% 3,3' diamidinobenzidine (DAB). The sections were then mounted on chrome-coated glass slides, air-dried, and washed with tap water. Finally, tissues were dehydrated in a graded series of ethanol solutions, cleaned in xylene, and covered with Entellan (Merck, Darmstadt, Germany).

RATIO OF MOTONEURONS WITH CGRP IMMUNOREACTIVITY FOR EACH MUSCLE

Muscle	*Mean ± SD (%)*
Cricothyroid muscle (n = 3)	93.0 ± 0.6
Thyroarytenoid muscle (n = 5)	71.4 ± 8.2
Posterior cricoarytenoid muscle (n = 5)	85.5 ± 3.5

RESULTS

The CTBG-labeled motoneurons (CTBG MNs) were clearly identified by the presence of scattered black grains in their cytoplasm, and the CGRP MNs were identified by the presence of brown DAB products in their cytoplasm. The CTBG-labeled neurons without CGRP immunoreactivity and the CTBG-labeled neurons with CGRP immunoreactivity (CTBG-CGRP MNs) were clearly identified, since the black grains resulting from silver intensification of CTBG were clearly distinguishable from the DAB products obtained by immunohistochemistry (see Figure). No differences in size or morphology were detected between CGRP-immunoreactive neurons and CGRP-negative neurons.

The CTBG MNs from the CT muscle were located in the ventromedial division of the rostral part of the NA. The number of CTBG MNs varied from 123 to 221. Most of the CTBG MNs had CGRP immunoreactivity, and the percentage of CTBG-CGRP MNs did not vary among the 3 dogs (range 92.7% to 93.7%). The CTBG MNs from the TA muscle were located in the dorsal division of the medial part of the NA. The number of CTBG MNs varied from 115 to 200. The CTBG-CGRP MNs showed no particular distribution among the CTBG MNs. The percentage of CTBG-CGRP MNs varied from 61.0% to 82.6%. The CTBG MNs from the PCA muscle were located in the ventral division of the medial part of the NA. The number of CTBG MNs varied from 122 to 210. The CTBG-CGRP MNs showed no particular distribution among the CTBG MNs. The percentage of CTBG-CGRP MNs varied from 78.8% to 88.6%. The Table summarizes the results from each muscle.

DISCUSSION

Llewellyn-Smith et al[8] introduced CTBG as a retrograde tracer that can be used in combination with light and electron microscopic immunohistochemistry. Although most of the usual tracers can be used in combination with immunohistochemistry, many have various disadvantages that render them inappropriate for certain types of studies.[8] We have already described the usefulness of this tracer in combination with immunohistochemistry for the investigation of the innervation system of the larynx and pharynx.[7,9,10]

Since Rosenfeld et al[2] first reported the presence of CGRP MNs in the NA, other researchers have subsequently confirmed that not all of the motoneurons in the NA contain CGRP.[3-5] Stuesse and Sawchenko[3] evaluated the percentage of neurons retrogradely labeled with CGRP in the rat NA by combining fluorescent labeling and CGRP immunohistochemistry, and found the following frequencies of CGRP-positive neurons: cardiac 100%, cervical esophagus 98%, abdominal esophagus 83%, abdominal vagi 92%, recurrent laryngeal nerve 84%, and gastric 71%. We similarly clarified the distinct percentage of CGRP MNs for the canine inferior pharyngeal constrictor muscle using CTBG as a tracer: thyropharyngeal muscle 70.3% and cricopharyngeal muscle 21.2%.[7] By contrast, McWilliam et al,[5] using horseradish peroxidase (HRP) as a tracer in combination with CGRP immunohistochemistry in cats, found that CGRP-like immunoreactivity was absent from motoneurons projecting into the viscera via the thoracic vagus, but was present in a large proportion (64%) of motoneurons with axons in the superior laryngeal nerve. They suggested that CGRP-like immunoreactivity was found only in vagal motoneurons that innervate striated muscle and that motoneurons without CGRP immunoreactivity originating from the superior laryngeal nerve were more probably preganglionic motoneurons projecting to the trachealis muscle, as the superior laryngeal nerve is known to provide efferent innervation of this muscle. Batten et al[4] also suggested that CGRP-positive neurons in the NA may be branchiomotor in function, and that CGRP-negative neurons were visceromotor, suggesting that the cat NA neurons showed a much lower degree (67%) of coexistence of CGRP-like immunoreactivity and choline acetyltransferase–like immunoreactivity than the other, purely branchiomotor or somatomotor nuclei. However, we disagree with their hypothesis, since our previous study[7] and the present study confirmed the distinct existence of motoneurons without CGRP immunoreactivity innervating striated muscle, by applying the tracer to a single striated muscle rather than a nerve or an organ.

The function of CGRP in motor innervation has been discussed by several investigators. Takami et al[11] reported that CGRP may be a neuromodulator related to striated muscle contraction. New and Mudge[12] proposed that CGRP may be a motoneuron-derived trophic factor that acts to increase the synthesis of acetylcholine receptors at the vertebrate neuromuscular junction. Although differences in the function of CGRP-negative motoneurons and CGRP-positive motoneurons innervating the striated muscle remain to be established, it is still noteworthy that not all of the motoneurons innervating the intrinsic laryngeal muscle have CGRP immunoreactivity, and that the percentages of CGRP MNs are various between intrinsic laryngeal muscles that have different functions. These findings suggest that the innervation and/or neurotrophic mechanism related to CGRP differs in each intrinsic laryngeal muscle.

The physiological characteristics of various motor units have been demonstrated to be correlated with the histochemical enzyme profiles of the muscle fibers in the motor units.[13,14] It is generally accepted that the highest proportion of type I fibers is observed in the PCA muscle, with fewer type I fibers in the CT muscle, and the lowest proportion in the TA muscle.[15-17] The present findings show that the difference in proportions of CGRP MNs in the NA between individual intrinsic laryngeal muscles does not correlate with the proportion of muscle fiber types, as proportions were highest (93.0%) in the CT muscle, intermediate (85.5%) in the PCA muscle, and lowest (71.4%) in the TA muscle. This suggests that differences in proportion of CGRP MNs between individual intrinsic laryngeal muscles may not reflect differences in the physiological innervation mechanism of each muscle, since there is considerable evidence to indicate that both the physiological characteristics of the motor unit and the enzyme profiles of the associated muscle fibers reflect the pattern of activity of the motor unit.[18] The CGRP in the laryngeal motoneurons may be related to the neurotrophic mechanism used in each muscle and may play an important role in nerve regeneration in cases of recurrent laryngeal nerve paralysis.

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ENDOSCOPIC TRANSSEPTAL SPHENOPALATINE ARTERY LIGATION FOR INTRACTABLE POSTERIOR EPISTAXIS

AHMED EL-GUINDY, MD
TANTA, EGYPT

The rigid nasal endoscope was used through a transseptal approach to reach the sphenopalatine foramen and to ligate its artery in 9 patients with intractable posterior epistaxis. Immediate and complete cessation of the bleeding uniformly occurred, except in 1 case, in which there was persistent bleeding on endoscopic examination of the nasal cavity at the end of the procedure. The ligature was checked and the artery was reclipped. Thereafter, the patient's recovery was uncomplicated and free of further epistaxis. Endoscopic transseptal sphenopalatine artery ligation offers a reliable option in the treatment of intractable posterior epistaxis. The submucoperiosteal dissection reduces bleeding, shortens operation time, and allows relatively easy identification of the sphenopalatine foramen. The procedure allows direct positive control of the major vessel supplying the posterior nasal cavity. It avoids the complications associated with transantral and pterygopalatine fossa surgery.

KEY WORDS — nasal bleeding, nasal endoscopy, nasal septum, sphenopalatine foramen.

INTRODUCTION

The bleeding nose is a common problem that can endanger life. The condition becomes much more complex with posterior epistaxis. This may be defined as epistaxis in which the bleeding vessel cannot be visualized by anterior rhinoscopy. It is a challenging problem for the otolaryngologist–head and neck surgeon and is usually managed by nonspecific remedies such as posterior packing and balloon catheterization. These traditional methods require prolonged hospitalization and may be associated with serious side effects such as hypoxia, bacteremia, and even myocardial infarction.[1] Moreover, they are often ineffective, because the protrusion of the middle and inferior turbinates prevents direct pressure over a bleeding site deep in the meatus.[2]

A logical approach in posterior epistaxis is the localization and identification of the bleeding vessel, followed by its control. The major source of posterior nasal blood supply is the terminal branch of the internal maxillary artery (IMA), the sphenopalatine artery (SA). Chandler and Serrins[3] described transantral ligation of the IMA in the pterygopalatine fossa. This approach carries the risks of marked swelling of the cheek, analgesia of the upper lip, neuralgic pain over the cheek and palate, postoperative maxillary sinusitis, and significant arterial or venous bleeding during dissection of the pterygopalatine fossa.[4] Recurrent epistaxis occurs in 9.5% to 15% of patients. The cause of failure is often technical, with inability to identify the IMA and its terminal branches at the time of surgery.[5]

Ligation of the SA allows secure, positive control of the major vessel supplying the posterior nasal cavity and avoids complications associated with surgery in the pterygopalatine fossa. The rate of recurrent epistaxis is lower than with IMA ligation, since the vessel is occluded closer to the site of bleeding, precluding the development of collateral blood flow.[6,7] The SA enters the nose through the sphenopalatine foramen, which is bounded superiorly by the body of the sphenoid, anteriorly by the orbital process of the palatine bone, posteriorly by the sphenoidal process of the palatine bone, and inferiorly by the perpendicular plate of the palatine bone. It is oval, with a longer vertical diameter of 6.5 mm (ranging from 5.0 to 8.0 mm) and a shorter horizontal diameter of 4.5 mm (ranging from 3.0 to 7.0 mm). It is located at the posterior end of the superior meatus (85%) or the middle meatus (5%) or is bridged by the basilar lamina of the middle turbinate (10%). As the perpendicular plate of the palatine bone runs obliquely from behind forward, the plane of the foramen forms an angle with the sagittal plane, opening forward and ranging from 0° to 30°.[8] There are a variety of approaches for SA ligation, ie, the extraperiosteal transantral,[6,7,9,10] the transnasal,[11] and the transseptal.[12]

Simpson et al[9] and Winstead[6] described transantral ligation of the SA at the medial side of the sphenopalatine foramen, and White[7] described it at its lateral side in the pterygopalatine fossa. Again, this approach carries the same risk as does transantral IMA ligation.

Budrovich and Saetti[11] suggested a transnasal approach to reach the SA at the anterior edge of its foramen after medially displacing the middle turbi-

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From the Department of Otolaryngology, Tanta School of Medicine, Tanta, Egypt.
CORRESPONDENCE — Ahmed El-Guindy, MD, PO Box 355, Tanta 31111, Egypt.

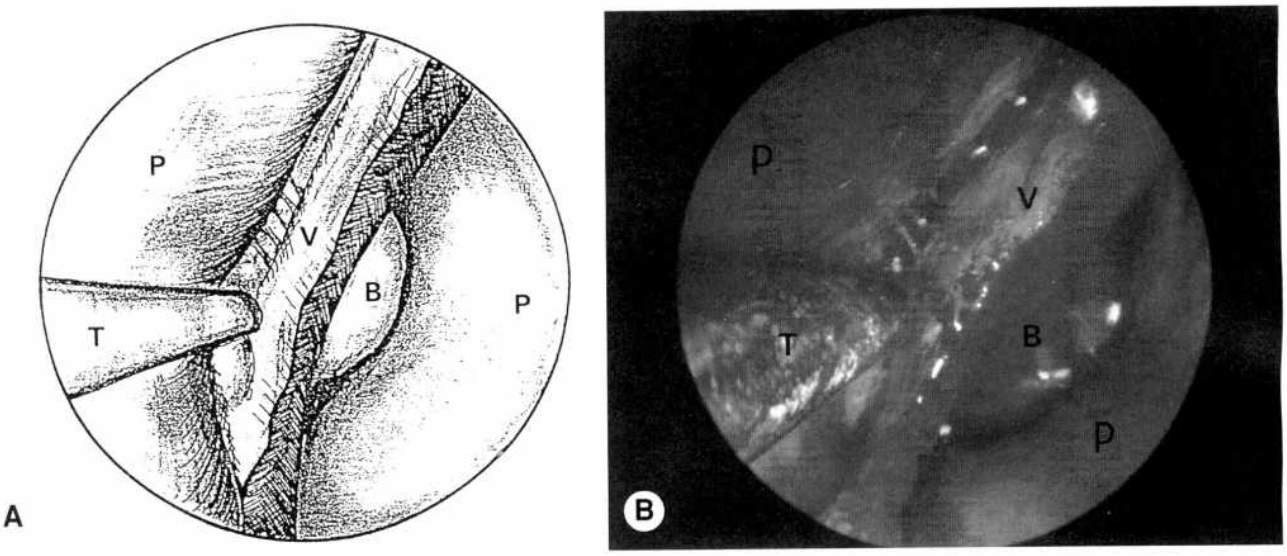

Fig 1. A) Diagrammatic representation and B) endoscopic illustration showing remnants of vomer (V) attached to rostrum of sphenoid. P — mucoperiosteal flap of septum; B — body of sphenoid; T — suction tip.

nates, but the middle turbinate almost always lies below the foramen. To reach the sphenopalatine foramen, it is necessary to section the posterior end of the middle turbinate or else to displace it laterally, not medially.[8] Moreover, there are many difficulties associated with this technique: a middle turbinate with a large posterior end, failure to find the sphenopalatine foramen at the expected site, bleeding from the mucosal incision, and unintentional penetration of the sphenoid or posterior ethmoid, as well as other problems such as deviated septum or multiple nasal polyposis.[13]

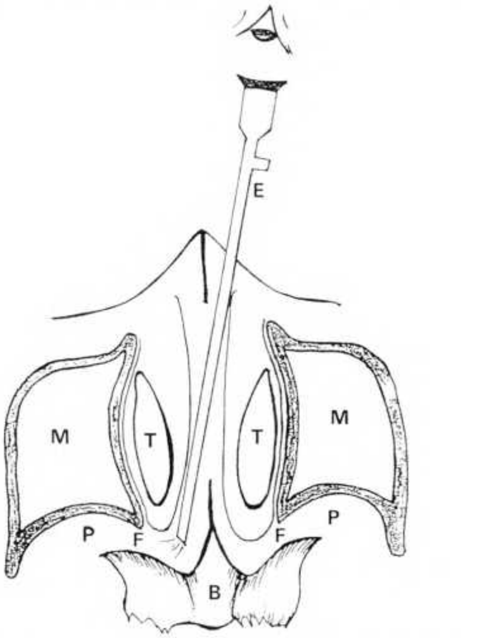

Fig 2. Diagrammatic representation showing how mucoperiosteal flap of septum is elevated off body of sphenoid (B) so that endoscope (E) inserted through one nostril into transseptal space can reach sphenopalatine foramen (F) of opposite side. M — maxillary sinus; T — middle turbinate; P — pterygopalatine fossa.

Minnis and Morrison[12] proposed the transseptal submucoperiosteal approach to reach the sphenopalatine foramen. This approach is rather blind; therefore, it contains some inherent risk of misdirection or overpenetration.

In this study, it was my intention to use the rigid nasal endoscope through a transseptal approach to ligate the SA under direct endoscopic vision.

PATIENTS AND METHODS

This study included 9 adult patients suffering severe posterior epistaxis who did not achieve hemostasis by the usual conservative methods, including endoscopic electrocautery. The male-to-female ratio was 2:1, and the ages ranged from 48 to 67 years. Informed consent was obtained before treatment began.

The procedure was performed with the patient under local anesthesia with neuroleptanalgesia. The rate of bleeding was reduced by insertion of a strip of cotton wool soaked in diluted Adrenalin. A hemitransfixion incision was made at the caudal rim of the septal cartilage, contralateral to the site of bleeding. The septal mucoperichondrium-mucoperiosteum was liberally elevated on both sides of the septum, back along the vomer to the body of the sphenoid. Parts of the cartilage and bony septum were removed as in submucous resection. For purposes of midline orientation, remnants of the vomer attached to the rostrum of the sphenoid were intentionally preserved (Fig 1).

A Storz rigid endoscope, 18 cm long, with 0° and

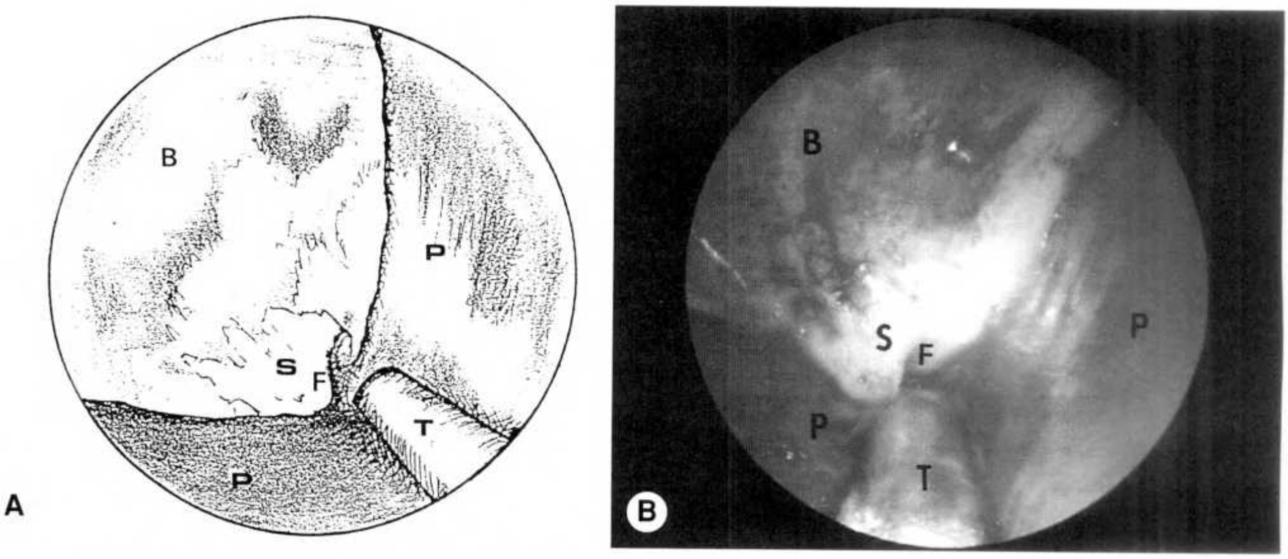

Fig 3. A) Diagrammatic representation and B) endoscopic illustration showing posterior rim of left sphenopalatine foramen (F). B — body of sphenoid; S — sphenoidal process of palatine bone; P — mucoperiosteal flap; T — suction tip.

30° deflection angles and a 4.0-mm external diameter, was used. The nasal endoscope was inserted through the contralateral naris into the transseptal space. Lateral mobilization of the mucoperiosteum was carried out, under endoscopic vision, over the anterior surface of the ipsilateral side of the body of the sphenoid, and then forward over the ipsilateral perpendicular plate of the palatine bone, by means of a suction dissector (Fig 2). As the flap was swept forward, the posterior rim of the ipsilateral sphenopalatine foramen was brought into view (Fig 3). It is the only well-defined opening in this area surrounded by solid bone. It is 12 mm above and lateral to the superior border of the posterior choana.[14] Dissection was continued forward along the superior and inferior margins of the foramen with medial traction on the nasal mucoperiosteum, until the sphenopalatine fibroneurovascular bundle was encountered (Fig 4), where the SA could be clipped or coagulated.

At the end of the procedure, the strips of cotton wool were removed, the nasal mucosa was replaced in its original position, the transfixion incision was sutured, and the nasal cavity was endoscopically examined for persistent bleeding. If there was none, the patient was recovered; otherwise, it would be necessary to check that the ligature had been correctly performed.

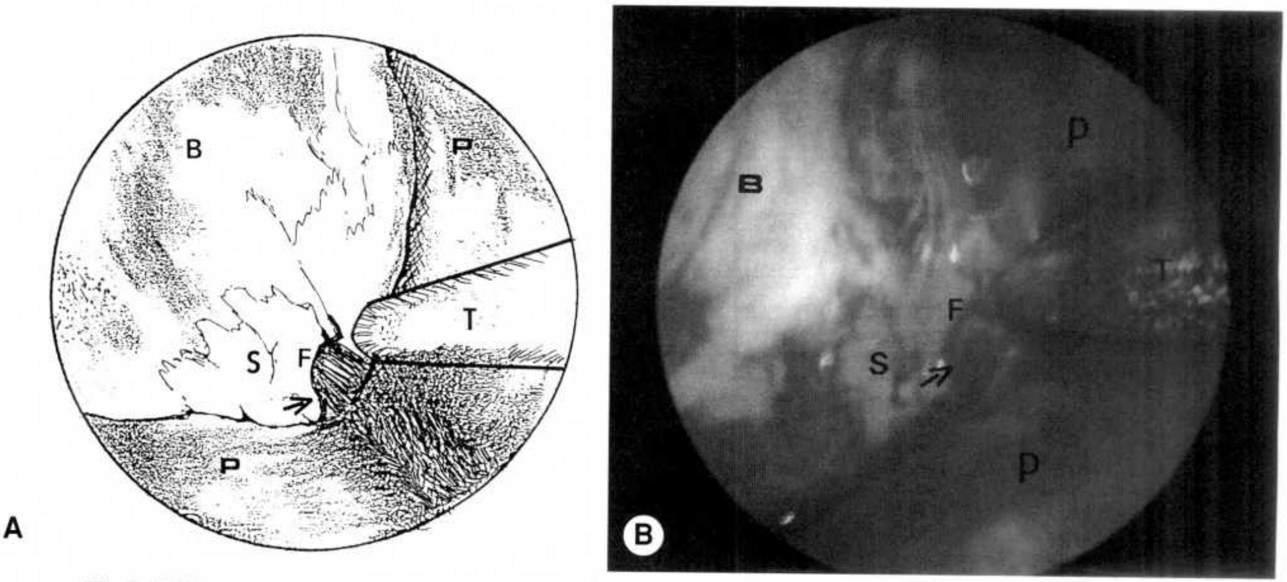

Fig 4. A) Diagrammatic representation and B) endoscopic illustration showing sphenopalatine artery (arrow) exiting sphenopalatine foramen (F). B — body of sphenoid; S — sphenoidal process of palatine bone; P — mucoperiosteal flap; T — suction tip.

RESULTS

With the nasal endoscope, it was possible to identify the sphenopalatine foramen and to reach the SA. The artery was ligated under direct endoscopic vision in all cases. Immediate and complete cessation of posterior epistaxis uniformly occurred, except in 1 case, in which there was persistent bleeding on endoscopic examination of the nasal cavity at the end of the procedure. The ligature was checked and the artery was reclipped. Thereafter, the patients' recovery was uncomplicated, and free of further epistaxis for a follow-up period of 3 to 6 months.

Great patience was often required to start the elevation of the mucoperichondrium at the caudal end of the septum; this being done, the remaining elevation was comparatively easy. However, mobilization of the mucoperiosteum over the perpendicular plate of the palatine bone was sometimes difficult because of fibrous attachment at the sphenopalatine suture. Sharp dissection against the bone allowed the surgeon to enter the subperiosteal plane without tearing the flap.

Endoscopic surgery is primarily a one-handed procedure. Initially, this one-handedness may appear as a handicap, but experience did not show this to be a problem. Again, one may encounter some difficulty in avoiding contact between the lens of the endoscope and the mucoperiosteal flap. Such contact requires frequent withdrawal, cleaning, and reintroduction of the endoscope.

The main operative difficulty encountered was a large posterior end of the middle turbinate. If it was too large to allow lateral mobilization of the mucoperiosteal flap over the anterior surface of the body of the sphenoid, it had to be sectioned.

DISCUSSION

Endoscopic transseptal SA ligation has several advantages over the approaches previously discussed. First, the dissection is submucoperiosteal along the anterior surface of the body of the sphenoid and the sphenoidal and orbital processes of the palatine bone. This shortens the operation time and reduces bleeding.

The transfixion incision and removal of parts of the cartilaginous and bony septum allows easy angulation of the endoscope so it can reach as far laterally as the dissection advances. This overcomes the difficulty of the narrow operative field that is sometimes encountered during surgical procedures on the retroturbinal area of the lateral nasal wall and caused by septal deviation, multiple nasal polyposis, and a large posterior end of the middle turbinate.

Additionally, the sphenopalatine foramen is easily localized because it is the only well-defined opening in the area surrounded by solid bone. Therefore, there is no possibility that the foramen will not be found or that the sphenoid or posterior ethmoid sinuses will be entered inadvertently.

The sphenopalatine foramen is approached along its posterior rim. This avoids injury to the SA, which is enclosed in a protective lamina as it crosses the anteroinferior margin of the foramen. There, the artery can be clipped under direct endoscopic vision. The nasal endoscope offers a more direct, close, and angled vision than the operative microscope.[6,7,10]

Bilateral procedures, if necessary, can be performed through a single approach. Endoscopic transseptal SA ligation can be used in cases of failure or rebleeding after packing, electrocautery, and transantral IMA ligation have been tried. I agree with White[7] that SA ligation is not an alternative to endoscopic electrocautery, but rather, an operation to complement it, available for cases in which hemostasis cannot be achieved.

The contraindication to this technique is previous submucous resection, which would prevent separation of the two layers of mucoperichondrium.

CONCLUSION

Endoscopic transseptal SA ligation offers a reliable option in the treatment of intractable posterior epistaxis. The submucoperiosteal dissection reduces bleeding and allows easy identification of the sphenopalatine foramen. The procedure allows direct, secure, positive control of the major vessel supplying the posterior nasal cavity. It avoids the complications associated with transantral and pterygopalatine fossa surgery. The rate of recurrent epistaxis is lower than in IMA ligation, since the vessel is occluded closer to the site of bleeding, precluding the development of collateral blood flow.

Endoscopic transseptal SA ligation is a minor surgical procedure with high efficacy and minimal postoperative morbidity. More cases and a longer period of follow-up are necessary to provide long-term results.

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TNM GLOTTIC: ROLE OF THE VOCAL MUSCLE, ARYTENOID CARTILAGE, AND INFERIOR PARAGLOTTIC SPACE IN IMPAIRED VOCAL CORD MOBILITY (T2)

AN EMBRYOLOGICAL AND CLINICAL STUDY

LUCIO RUCCI, MD
LUCIO GAMMAROTA, MD
LAURA SIMONETTI, MD
MARIA BRUNA BORGHI CIRRI, MD
FLORENCE, ITALY

The TNM classification is unsatisfactory regarding the impaired mobility of the vocal cord and/or the arytenoid. The authors have studied the intrinsic laryngeal musculature, with particular attention to the medial thyroarytenoid muscle, the lateral thyroarytenoid muscle, and the inferior paraglottic space. The study was conducted on serial sections of 4 embryonal and 9 fetal larynges at different stages of development. The embryological observations showed that the lateral and medial thyroarytenoid muscles have different maturation times and probably different functions: phonatory and sphincteric. The authors have found that the inferior paraglottic space is situated between these 2 muscles. It would represent a path of diffusion for glottic cancer and play a central role in impaired vocal cord and/or arytenoid mobility, according to the degree of diffusion. Embryological observations have suggested the existence of laryngeal morphofunctional units belonging to the subsites of the glottic region and related to tumoral spreading.

KEY WORDS — cancer, embryology, inferior paraglottic space, larynx, TNM classification.

INTRODUCTION

The TNM glottic cancer classification is tied to subjective evaluation criteria, both for vocal cord mobility and for T size. Therefore, TNM proves to be not always suitable for therapy choices and little adapted to multicentric comparison. As generally known, the TNM classification of laryngeal cancer, based on the criterion of the T extent in the sites and subsites and on the criterion of cordal mobility, makes objective reference neither to the dimensions of the tumor nor to the degree of infiltration (with the exception of the T4 stage, which takes cartilage invasion into account).

The latest unified draft of TNM 1989 (UICC, 1987,[1] and AJCC, 1988[2]) and the most recent editions of 1992[3,4] do not seem to have completely resolved the criticisms laid upon preceding editions. Some uncertainties still remain concerning the anatomic delimitation of the glottic region and of its subsites, the significance of impaired cordal mobility, the evaluation of diffusion pathways, and the structures involved in deep tumoral infiltration.

Conflicting opinions are found in the literature regarding the spatial extent of the glottic site and its subsites, whose boundaries are identified with reference to the anatomic structures that compose them. Some laryngologists consider the ventricular floor to be in the glottic region,[5] while other authors[6] assign the glottic region exclusively to the zone lined with stratified squamous epithelium, which thus includes the edges of the vocal cords, Reinke's space, the anterior commissure, and the medial face of the arytenoid cartilage.

The arytenoid, a subsite of the supraglottis for AJCC and UICC, is considered by many to be in the glottic region,[7,8] with consequent significant differences in classification and therapy. Further, the TNM definition of the posterior commissure is ambiguous, considered by some as being bordered by the space between the arytenoids, and by others as extending to the vocal process of the arytenoid and to the posterior extremity of the ventricular bands.[9]

The anterior commissure, the crucial point of neoplastic laryngeal pathology, needs separate consideration, since it requires an anatomic and clinical definition and a classification in TNM glottic for tumors involving this subsite.[10,11]

Having recognized the importance of correct delimitation of the glottic region or site, of the identification of its subsites, of the problem of cordal mo-

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From the Otorhinolaryngology Clinic (Rucci, Gammarota, Simonetti) and the Department of Human Anatomy and Histology, Section "E. Allara" (Borghi Cirri), University of Florence, Florence, Italy. This work was supported by the Italian Ministry of University and Scientific and Technology Research ("University Funds 40%") and by Regione Toscana (as a part of the Third Project of Finalized Health Research; grant 940195).

CORRESPONDENCE — Maria Bruna Borghi Cirri, MD, Dept of Human Anatomy and Histology, Section "E. Allara," Viale G. Pieraccini 6, I-50139 Firenze, Italy.

DESCRIPTION OF SPECIMENS

Quantity	*Classification*	*Development Stage (wk)*	*Crown-Rump Length (mm)*
4	Embryos	7-8	25-30
2	Fetuses	11	45-50
1	Fetus	15-16	70
2	Fetuses	20	167-170
1	Fetus	23	120
2	Fetuses	33	310-315
1	Fetus (stillborn)	36-37	340

bility in all its aspects, and of the diffusion pathways taken by glottic cancer, we wished to reconsider the structures of this site and to reevaluate the TNM classification criteria on the basis of a careful embryological and anatomic study of the region, integrated with anatomic-pathologic and clinical observations of glottic carcinoma.

MATERIALS AND METHODS

The research was conducted on serial sections of larynges from 4 embryos and 9 fetuses. The Table summarizes the embryonal and fetal specimens employed.

The embryos and fetuses, except for that of the 37th week, resulted from legal abortions and did not exhibit malformative disorders of the larynx. The ethical guidelines of Italian law and the Italian National Medical Council were followed.

The embryonal and fetal specimens were fixed in 10% phosphate-buffered formol and embedded in paraffin. Serial sections were stained with hematoxylin and eosin and with Van Gieson's stain.

RESULTS

Weeks 7 and 8 (Crown-Rump Length, 25 to 30 mm). In the 7- to 8-week embryos (Fig 1A), the laryngeal cartilages are represented by the 2 lateral laminae of the thyroid cartilage undergoing chondrification, by the mesenchymal intermediate lamina, and dorsally by the arytenoids. The latter are made up of cartilaginous tissue in the dorsal-lateral portion and of mesenchyme in the lateral-ventral portion, from which the vocal process develops.

The glottic space assumes a T-shape; in fact (Fig 1B), posteriorly, it is long and narrow, in correspondence with the arytenoid cartilages, while anteriorly, it has a transverse major axis, in correspondence with the primordium of the vocal muscles.

The interarytenoid muscle is represented by myotubes and fibers, while the posterior cricoarytenoid muscle is made up predominantly of myotubes situ-

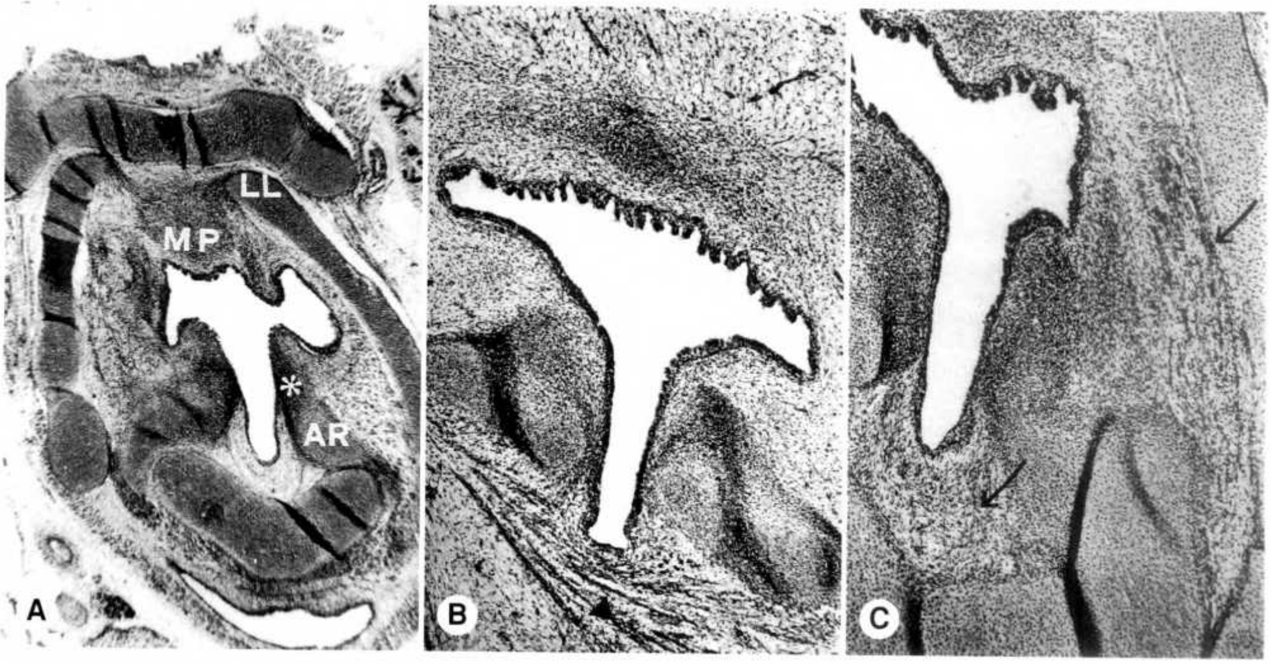

Fig 1. Seven- to 8-week embryos (crown-rump length, 25 to 30 mm). A) Transverse section of larynx, above glottic plane (H & E, original ×30). Lateral laminae (LL) made of cartilage tissue, primordium of intermediate lamina of thyroid cartilage (still formed of mesenchyme), and median process (MP) are visible. Posteriorly, arytenoids (AR) are visible and continue, ventrally, with mesenchymal primordium of vocal process (asterisk). Primordium of posterior cricoarytenoid muscle can be seen. B) Transverse section of larynx, above glottic plane (H & E, original ×75). Glottic space has T-shape. Dorsally, primordium of interarytenoid muscle is visible, formed of fibers and myotubes (triangle). C) Transverse section of larynx, above glottic plane (H & E, original ×75). Arrows show primordium of lateral thyroarytenoid muscle and, posteriorly, at level of cartilaginous glottis, primordium of internal cricoarytenoid ligaments, 1 per side.

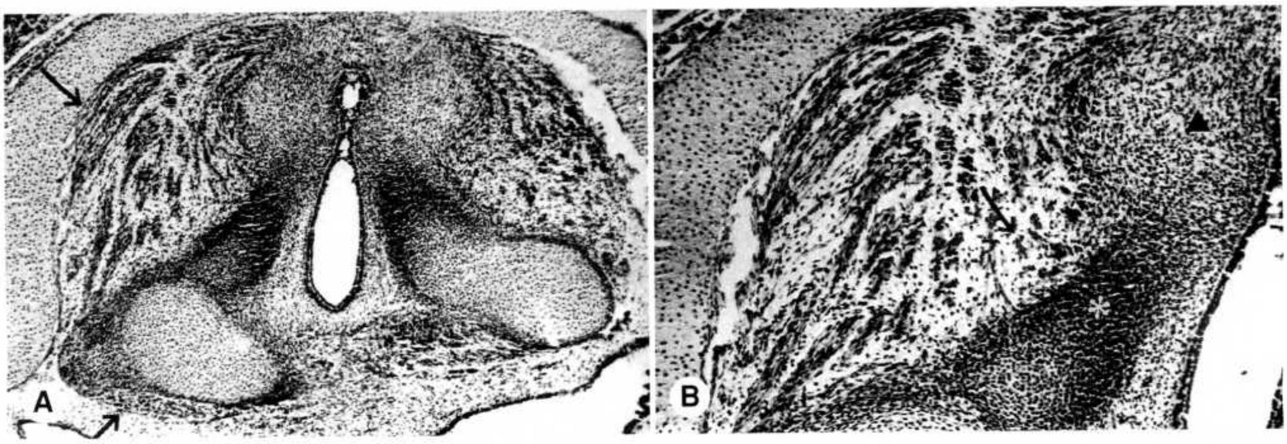

Fig 2. Eleven-week fetuses (crown-rump length, 45 to 50 mm). A) Transverse section of larynx, just below glottic plane (H & E, original ×30). Shows interarytenoid muscle fibers, posterior cricoarytenoid muscle (arrows), part of lateral thyroarytenoid muscle fibers, and myotubes of medial thyroarytenoid muscle. B) Transverse section of larynx, just below glottic plane (H & E, original ×75). Vocal process (asterisk), formed by mesenchyme, continues forward with part of mesoderm (triangle), surrounded at its periphery by myotubes constituting primordium of medial thyroarytenoid muscle. Arrow indicates thin mesenchyme that will make up future inferior paraglottic space.

ated along the dorsal margins of the arytenoid cartilage.

In the lower sections (Fig 1C) of the glottic region, some myoblasts and myotubes are visible and constitute the outermost fibers of the lateral thyroarytenoid muscle. Further, the mesenchymal primordium of the vocal process extends anteriorly, with consequent lengthening of the vertical branch of the "T" glottic space. Dorsally, 2 well-defined contingents of mesenchyme, 1 on each side, surround the arytenoid and cricoid cartilages. They are at the sides of the cartilaginous glottis in the proximity of cricoarytenoid articulation and give rise to, in our opinion, ligamentous structures that we identify as internal cricoarytenoid ligaments.

In this early phase of development, maturation proceeds caudal-cranially and dorsal-lateral-ventrally for most of the intrinsic musculature.

Week 11 (Crown-Rump Length, 45 to 50 mm). In the 11-week fetus, the thyroid cartilage is completely formed, the lateral laminae are joined with the intermediate lamina, and only the primordium of the vocal process of the arytenoid cartilage is still made up of mesenchyme.

During this period, the intrinsic muscles of the larynx (Fig 2A) are represented by the interarytenoid muscle, the posterior cricoarytenoid muscle, the lateral thyroarytenoid muscle, and the myotube-based outermost fibers of the medial thyroarytenoid or vocal muscle. The 2 components, lateral and medial, of the thyroarytenoid muscle are distinguishable by the different orientation of the elements that form them.

The vocal process of the arytenoid (Fig 2B), still made up of a mesenchymal primordium, continues anteriorly with a mesodermic contingent. This contingent shows some myotubes at the periphery; these are the outermost structures of the maturing vocal muscle.

Maturation of the mesodermic-mesenchymal primordia is correlated with the modification of the glottic space; in fact, in the upper sections with less mature primordia, the glottic space is smaller, while in the lower sections with more mature primordia, the glottic space extends into a split with an anterior-posterior major axis.

The mesenchymal tissue, which, according to our observations, is the future site of the inferior paraglottic space (IPS), is delimited by the outermost fibers (Fig 2B) of the medial thyroarytenoid muscle and the innermost fibers of the lateral thyroarytenoid muscle. These fibers are arranged in bundles having different slopes. This space separates 2 muscular contingents in the thyroarytenoid muscle, 1 lateral and the other medial, with different maturation times and thus, in our view, correlatable with different functions.

In the 11th week (Fig 2), the joint observation of serial sections of the glottic region has allowed us to emphasize that the maturation of the larynx's intrinsic musculature proceeds by fanning out around the arytenoids in a dorsal-lateral-ventral direction, while the maturation of the cartilaginous arytenoids, the vocal process, and the vocal muscle is realized in a dorsal-ventral direction and simultaneously, for the vocal muscle, also from the outside in. The entire maturation system, moreover, proceeds from caudal to cranial.

Weeks 16 and 17 (Crown-Rump Length, 70 mm). In the inferior portion of the glottic region (Fig 3A),

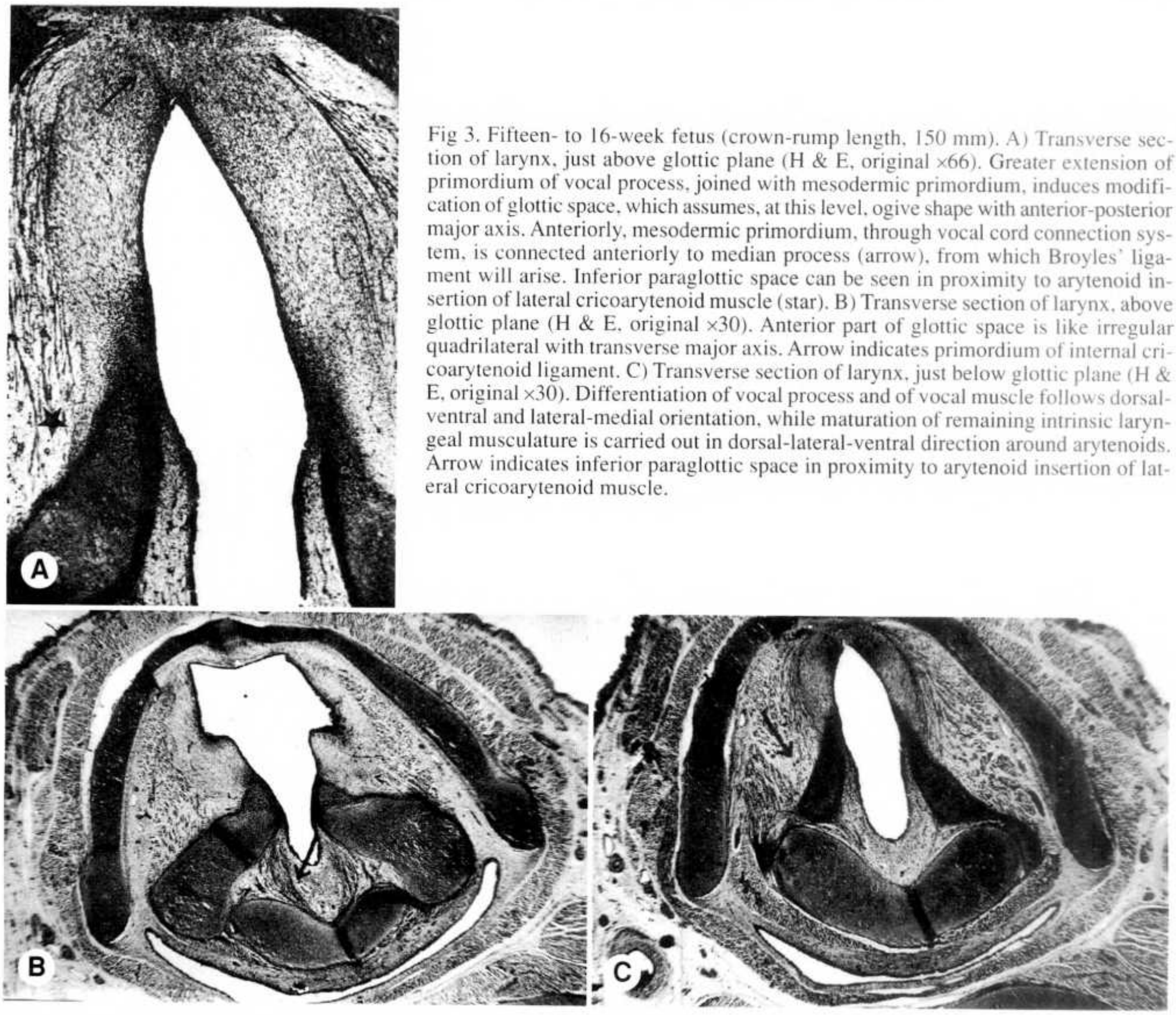

Fig 3. Fifteen- to 16-week fetus (crown-rump length, 150 mm). A) Transverse section of larynx, just above glottic plane (H & E, original ×66). Greater extension of primordium of vocal process, joined with mesodermic primordium, induces modification of glottic space, which assumes, at this level, ogive shape with anterior-posterior major axis. Anteriorly, mesodermic primordium, through vocal cord connection system, is connected anteriorly to median process (arrow), from which Broyles' ligament will arise. Inferior paraglottic space can be seen in proximity to arytenoid insertion of lateral cricoarytenoid muscle (star). B) Transverse section of larynx, above glottic plane (H & E, original ×30). Anterior part of glottic space is like irregular quadrilateral with transverse major axis. Arrow indicates primordium of internal cricoarytenoid ligament. C) Transverse section of larynx, just below glottic plane (H & E, original ×30). Differentiation of vocal process and of vocal muscle follows dorsal-ventral and lateral-medial orientation, while maturation of remaining intrinsic laryngeal musculature is carried out in dorsal-lateral-ventral direction around arytenoids. Arrow indicates inferior paraglottic space in proximity to arytenoid insertion of lateral cricoarytenoid muscle.

the greater ventral extension of the vocal process of the arytenoids, and of the mesoderm with which it is continuous, induces modifications in the glottic space, which at this level assumes an ogive shape with an anterior-posterior major axis. In the upper sections (Fig 3B) of the glottic region, the space has an irregular quadrilateral shape with a transverse major axis, owing to less advanced maturation of the mesoderm in the lateral-medial direction. One further observes, in the proximity of the roots of the vocal process, 2 contingents of a connective nature that extend, 1 on each side, from the anterior surface of the cricoid to the posterior face of the arytenoids, surrounding the mucosa of the cartilaginous glottis. These 2 structures, which surround the last caudal bundles of the interarytenoid muscle, constitute the internal cricoarytenoid ligaments, in relation with the cricoarytenoid articulation, and are already evident in the 8th week (Fig 1C).

In the glottic region, the thick mesoderm (Figs 1A and 3A) that follows the mesenchymal primordia of the vocal process extends forward until it connects with the median process via the vocal cord connection system. In the outermost portion, this mesoderm shows arching muscular elements with an anterior-posterior course that are in contact with the lateral thyroarytenoid muscle fibers. These are the outermost fibers of the medial thyroarytenoid muscle or vocal muscle, while the more medial mesodermic component begins to differentiate itself into mesenchyme.

Further, in this period (Fig 3C) one recognizes the lateral thyroarytenoid muscle and some fibers of the medial thyroarytenoid muscle, distinguishable from each other because of their different orientations and insertion points and the presence of the IPS in between. One can also recognize the posterior and lateral cricoarytenoid muscles.

The notable datum in the 16th week is that the morphologic aspects of the intrinsic muscles and of

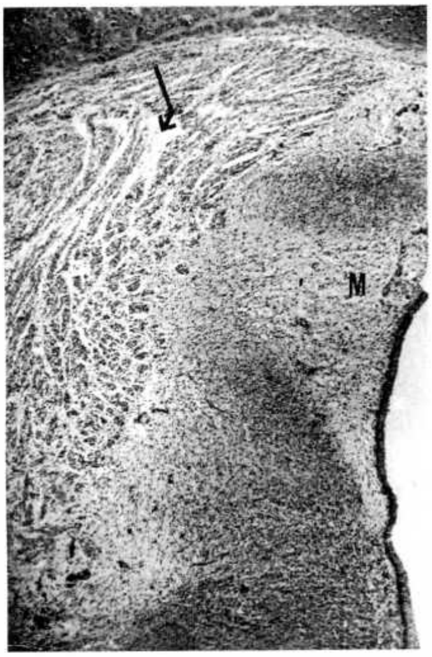

Fig 4. Twenty-week fetus (crown-rump length, 160 to 170 mm; H & E, original ×66). Transverse section of larynx, just above glottic plane. Vocal process continues ventrally with mesodermic part that surrounds zone of mesenchyme (M) from which originate lamina propria and connective structures attached to vocal muscle. Mesodermic part proceeds ventrally with vocal cord connection system. Arrow indicates connective shoot from which inferior paraglottic space originates.

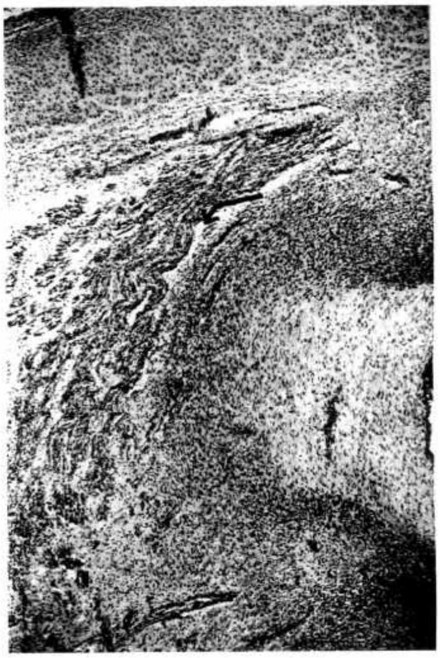

Fig 5. Twenty-three-week fetus (crown-rump length, 180 to 200 mm; H & E, original ×66). Transverse section of larynx, above glottic plane. Arrow indicates inferior paraglottic space, which separates maturing medial thyroarytenoid muscle from already-differentiated lateral thyroarytenoid muscle.

the laryngeal cartilages reinforce the concept that the differentiation proceeds in a dorsal-ventral direction for the vocal muscle and process, and dorso-lateral-ventrally around the arytenoids for the remaining intrinsic musculature.

Week 20 (Crown-Rump Length, 167 to 170 mm). In the 20th week of intrauterine life, the intrinsic muscles of the larynx are almost fully differentiated; the interarytenoid muscle, the lateral thyroarytenoid muscle, and the outermost fibers of the medial thyroarytenoid or vocal muscle are easily recognizable, as are the posterior and lateral cricoarytenoid muscles (Fig 3C).

The mesenchymal primordium (Fig 4) of the vocal process continues forward with a mesodermic contingent that surrounds a medially placed mesenchymal component, from which originate, according to our observation, the connective structures of this region (the vocal ligament, Reinke's space, the lamina propria of the laryngeal mucosa, etc).

The mesodermic contingent (Fig 4) from which the vocal muscle develops is maturing, as the presence of myoblasts, myotubes, and muscular fibers shows. This contingent, through the vocal cord connection system, is in contact ventrally with the median process, from which Broyles' ligament will develop (Fig 3A).

Anteriorly (Fig 4), between the lateral and medial thyroarytenoid muscles, a thin connective stem remains that in our view represents the primordium of the IPS and that separates the vocal muscle from the lateral thyroarytenoid muscle (Fig 3C), both in anterior-posterior and cranial-caudal directions.

In the lower laryngeal sections, at the level of the arytenoid body (Fig 3B), the further-differentiated internal cricoarytenoid ligaments are evident. The presence of this differentiating mesodermic and mesenchymal component attests to the difference of the vocal muscle in relation to the remaining intrinsic laryngeal musculature, and in particular to the lateral thyroarytenoid muscle.

Week 23 (Crown-Rump Length, 120 mm). In the 23rd week, the muscles of the larynx appear in a more advanced state of differentiation. The vocal process (Fig 5) continues anteriorly with the subepithelial mesenchymal component surrounded by the mesodermic contingent, which, projecting ahead, enters into continuity with the maturing connection system of the vocal cords (Fig 6A). A discrepancy exists, however (Figs 3A,C, 4, and 5), between the development of the lateral thyroarytenoid muscle, completely differentiated into striated muscular fibers, and the medial thyroarytenoid muscle, still made up of a mesodermic primordium containing muscular

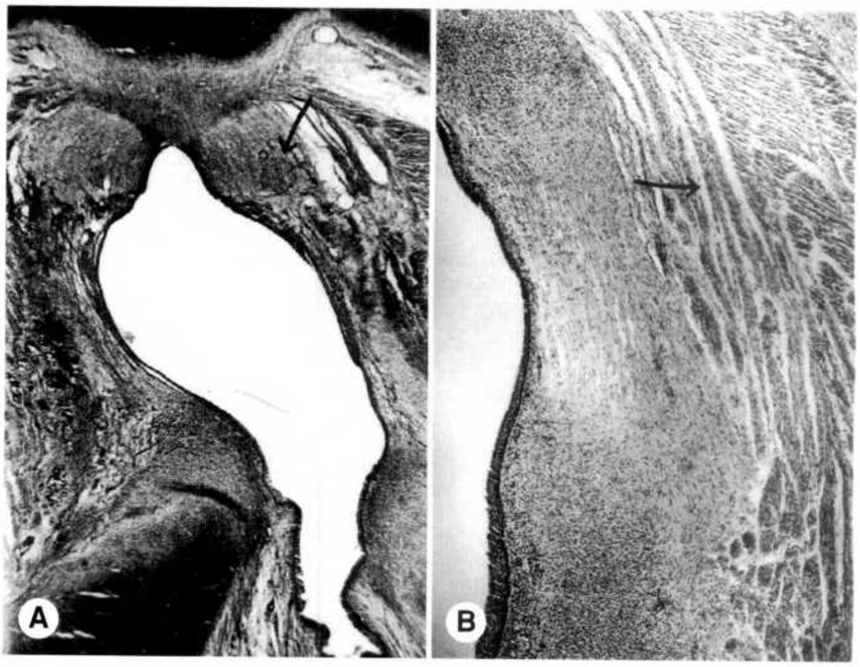

Fig 6. Thirty-three-week fetuses (crown-rump length, 310 to 315 mm). A) Transverse section of larynx, above glottic plane (H & E, original ×20). Arrow indicates one of elastic noduli that have been differentiated in vocal muscles' connection. B) Transverse section of larynx, above glottic plane (H & E, original ×66). Vocal process has grown further in ventral direction and is connected to mesenchyme from which connective components associated with vocal muscle will originate. Arrow shows inferior paraglottic space.

fibers at the periphery and surrounded by a medial mesenchymal contingent. A thin connective tissue layer that is already present at the 11th week (Fig 2B) is interposed between these 2 structures, both of which have different maturation times and probably different functions. This connective tissue becomes prominent at the 20th week (Figs 3A,C and 6A) and is now (Fig 5) well delimited anteriorly and identifiable with the IPS, which runs in an anterior-posterior direction.

In this period, the primordium of the vocal process, the IPS, and the lateral thyroarytenoid muscle are distinct both in maturation times and in position, while the vocal process–vocal muscle complex (Figs 3C, 4, and 5) constitutes, in our opinion, a separate entity with its own differentiation timetable.

Week 33 (Crown-Rump Length, 310 to 315 mm). The outermost part of the primordium of the medial thyroarytenoid muscle is made up of myotubes and muscular fibers; medially, at the primordium, one observes a contingent made of mesenchyme, which is the origin of the vocal ligament and connective structures such as Reinke's space, etc (Fig 5).

In the context of the vocal muscle connection system (Fig 6A), in strict continuity with Broyles' ligament, derived from the dorsal part of the median process, the elastic nodule complex appears; this is connected to the vocal muscle, which is now undergoing organization in accordance with its specialized function.

In the lower sections of the glottic region (Fig 6B), the vocal process elongates, joined with the medial mesenchymal primordium from which the connective component (vocal ligament and connective structures) originates. This mesenchymal component is included between the lamina propria and the innermost bundles of the vocal muscle. This muscle is now greatly developed and differentiated.

This confirms the fact that the intrinsic muscular maturation is carried out mainly in a dorsal-lateral-ventral direction, while the maturation of the arytenoid, vocal process, vocal muscle, and vocal cord connection system is realized predominantly in a dorsal-ventral direction. Moreover, the entire maturation of the larynx proceeds in a caudal-cranial direction.

In this stage of development, all those muscles that insert into the arytenoids (Fig 3A,C) have reached a more advanced stage of maturation, fanning out around these cartilages from back to front, together with the internal cricoarytenoid ligaments (Fig 3B). Together, they ensure a sphincteric function. The maturation of the connective component (Fig 6B), represented by the vocal ligament and its related structures and by the vocal process, is incomplete at this stage.

In this period, the medial thyroarytenoid muscle appears well separated from the lateral thyroarytenoid muscle due to the IPS, which extends anterior-posteriorly, even in proximity (Fig 3A,C) to the arytenoidal insertion point of the lateral cricoarytenoid muscle.

Weeks 36 and 37 (Crown-Rump Length, 340 mm). Even in the full-term fetus, it is possible to notice that the lower parts of the larynx are in a more advanced stage of differentiation than are the upper parts.

All of the intrinsic muscles are completely formed except for the vocal muscle, which is mature only in its outer part. The vocal process is continuous anteriorly with a connective tissue from which the vocal ligament differentiates. The vocal process is included between the lamina propria and the innermost fibers of the medial thyroarytenoid muscle, a muscle now well distinct from the lateral thyroarytenoid muscle, owing to the direction of its fibers, as well as to the presence of the IPS.

In the full-term fetus, one observes a picture almost superimposable upon that described for the 23rd week.

The arytenoids, which represent a point of insertion for the larynx's intrinsic musculature and ligament structures, can be considered a center of phylogenetic organization for all the muscular and fibrous structures that are strictly correlated with both sphincteric and phonatory functions of the larynx.

The IPS, besides dividing the lateral thyroarytenoid muscle (already organized into bundles) from the medial one (represented by a unique muscular contingent or vocal muscle), would separate the structures with a mainly sphincteric function from those with a mainly phonatory function.

DISCUSSION

Our observations lend themselves to the following morphologic and functional considerations. The intrinsic musculature of the larynx essentially shows 2 different maturation times. The interarytenoid, posterior cricoarytenoid, lateral cricoarytenoid, and lateral thyroarytenoid muscles, together with the internal cricoarytenoid ligaments (ie, all structures correlated to sphincteric function), show an early maturation that proceeds around the arytenoid, in a fan-like orientation, in a dorsal-lateral-ventral direction. The vocal muscle, with a mainly phonatory function, together with the underlying mesenchyme from which originate parts of the lamina propria of the laryngeal mucosa, the vocal ligament, Reinke's space, etc, shows a deferred and late maturation, still incomplete at birth. The maturation of the vocal muscle and of its correlated structures begins, according to our observation, from the vocal process of the arytenoid and proceeds predominantly in a posterior-anterior direction and, to a lesser degree, in a lateral-medial one.

The lateral thyroarytenoid muscle and the vocal muscle would delimit the IPS, extended in a ventral-dorsal direction from the anterior commissural region to the arytenoidal insertion point of the lateral cricoarytenoid muscle. This connective space would represent the anatomic-functional separation of the vocal muscle from the remaining intrinsic musculature, the former with a mainly phonatory function, the latter with a function predominantly sphincteric. The IPS, in our view, constitutes a functional buffer of loose connective tissue necessary for the sliding of the vocal muscle.

The entire vocal process–vocal muscle would take part in a morphofunctional unit or complex of the glottic region, together with the IPS, which represents the external border of this unit. The IPS would constitute a cordal cancer diffusion path toward the lateral thyroarytenoid muscle, the lateral cricoarytenoid muscle, the muscular process of the arytenoid, the subglottic region, and the anterior commissural region, with consequent functional modifications in the involved structures.

According to the concept of the morphofunctional units, the subdivision of the glottic site into subsites would tie the anatomic to the clinical data. In the glottic site, we have identified the following morphofunctional units or complexes:

1. Complex: vocal cord connection system, Broyles' ligament, and intermediate lamina.[10]
2. Complex: vocal process, vocal muscle, and IPS.
3. Complex: vocal process, arytenoid body, and internal cricoarytenoid ligaments.

The precise identification of the IPS between the lateral and the medial thyroarytenoid muscles allows us to correlate such a space to the normal cordal mobility and particularly to the sliding of the vocal muscle along the fibers of the lateral thyroarytenoid muscle. The latter, besides having a predominantly sphincteric role, would have, in our opinion, the function of supplying an appropriate support for the vocal muscle, thus cooperating in phonation. The role that we attribute to the IPS is confirmed by the constant cordal hypomobility (T2) observed in cases of partial infiltration of the vocal cord.[12,13]

This embryological study would enable a different view of the glottic region[14] with its identification of morphofunctional units or complexes, as well as of tumoral diffusion paths. The identification of the IPS would explain the impaired vocal cord mobility in glottic cancer.

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RHINOLOGY SYMPOSIA

The 18th International Symposium on Infection and Allergy of the Nose (ISIAN), the 4th Asian Research Symposium on Rhinology (ARSR), and the 3rd ASEAN Rhinology Group Congress (ARC) will be held in Manila, Philippines, February 14-18, 1999. For information, contact Gil M. Vicente, MD, Congress President, Dept of ORL-HNS, Jose Reyes Memorial Medical Center, Rizal Avenue Sta. Cruz, Manila, Philippines 1003; telephone (632) 743-6921; fax (632) 732-1077 or (632) 522-0946; e-mail isian@psohns.com; web site http://www.psohns.com/isian.

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TREATMENT OF BENIGN POSITIONAL VERTIGO USING HEELS-OVER-HEAD ROTATION

JOSEPH M. FURMAN, MD, PHD

STEPHEN P. CASS, MD, MPH

BRIAN C. BRIGGS

PITTSBURGH, PENNSYLVANIA

Particle repositioning, using either bedside techniques or whole-body manipulation devices, has been used effectively to treat benign positional vertigo (BPV). We assessed the efficacy of particle repositioning using a device designed and built specifically to treat BPV that rotated patients 360° heels-over-head in their sagittal body plane while their heads were turned to align the posterior semicircular canal with the plane of rotation. Eye movements were monitored during the maneuver by an infrared video recording system that allowed subsequent review of the induced nystagmus. Our results indicate that 1) heels-over-head rotation is an extremely efficacious procedure for treating patients with BPV and 2) the pattern of nystagmus during repositioning is consistent with the theory that free-floating debris is highly likely to account for BPV.

KEY WORDS — nystagmus, otoliths, vestibulo-ocular reflex.

INTRODUCTION

Benign positional vertigo (BPV) is a common otologic disorder characterized by brief episodes of vertigo induced by changes in head position. Compelling evidence exists that BPV is caused by free-floating debris in the endolymphatic space of the semicircular canals,[1] most commonly the posterior semicircular canal. This concept, first suggested by Hall et al[2] in 1979, has been corroborated by intraoperative observation of debris[1] and by the successful treatment of BPV using "particle repositioning maneuvers" (PRMs) designed to reposition the free-floating debris into the vestibule.[3,4] The origin of this debris, its chemical composition, and the flow characteristics of the debris within the endolymph are unknown. Some recent data suggest that spontaneous remission of BPV may be based on the solubility of the otoconia.[5] The most commonly used PRM is based on a technique, first described by Epley[3] in 1992, that is performed at the bedside and is designed to reposition debris out of the posterior semicircular canal. Monitoring eye movements during particle repositioning has been espoused as a useful adjunct to ensure that a successful maneuver has been performed.[4] Such a technique may help determine the optimal velocity of the head during particle repositioning. Clinical challenges concerning the use of PRMs for BPV include unexplained treatment failure, recurrence over time, and difficulty performing a PRM on patients who are obese or who have significant musculoskeletal problems such as cervical or lumbar vertebral disease that limits their motion. In particular, any musculoskeletal abnormality that limits head extension is likely to reduce the efficacy of bedside maneuvers based on the Epley technique.

A type of PRM that differs from the Epley technique, in that it uses sagittal-plane heels-over-head rotation, was described by Brey et al[6] in 1994 and by Lempert et al[7] in 1996 and is being used by J. M. Epley (personal communication). We report herein our experience using a heels-over-head sagittal body plane rotation device designed and built at the University of Pittsburgh. The rationale for our study was that we wished to assess the efficacy of particle repositioning using an optimal method for maximizing the use of gravity to pull endolymphatic debris out of the posterior semicircular canal and into the vestibule. Heels-over-head rotation using our custom-fabricated device enabled us to perform a reproducible maneuver while monitoring eye movements. These eye movement recordings allowed us to draw several inferences regarding the movement of endolymphatic debris during the diagnosis and treatment of BPV. An additional motivation for our study was the development of an alternative repositioning maneuver for those patients unable to tolerate the bedside Epley maneuver, eg, as a result of an inability to extend the head.

METHODS

This report describes our experience with 11 patients using sagittal body plane heels-over-head rota-

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From the Department of Otolaryngology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania. This work was supported in part by grant DC01791 from the National Institutes of Health.

CORRESPONDENCE — Joseph M. Furman, MD, PhD, Dept of Otolaryngology, The Eye & Ear Institute Building, 200 Lothrop St, Suite 500, Pittsburgh, PA 15213.

TABLE 1. CLINICAL CHARACTERISTICS OF PATIENTS

Patient No.	Sex	Age	Cause	Duration of Symptoms Prior to PRM	Vestibular Laboratory Results ENG	Rotation	Posturography
1	F	39	Posttraumatic	2 d	N/A	N/A	N/A
2	M	37	Postneuritis	4 mo	PN (L)	N/A	N/A
3	F	73	Idiopathic	4 mo	N/A	N/A	N/A
4	F	62	Idiopathic	5 mo	N/A	DP (L)	N/A
5	F	47	Posttraumatic	6 mo	NL	N/A	N/A
6	F	78	Posttraumatic	10 y	NL	NL	VP
7	F	61	Idiopathic	6 mo	SN (L)	DP (L)	VP
8	M	73	Idiopathic	5 y	NL	N/A	N/A
9	M	60	Idiopathic	3 mo	NL	NL	N/A
10	M	76	Idiopathic	2 mo	N/A	N/A	N/A
11	F	38	Idiopathic	2 wk	N/A	N/A	N/A

PRM — particle repositioning maneuver, ENG — electronystagmography, N/A — not available, PN — positional nystagmus, (L) — left-beating, DP — directional preponderance, NL — normal, VP — vestibular pattern, SN — spontaneous nystagmus.

tion as a novel treatment for BPV. Each of the 11 patients was identified through the practices of the Department of Otolaryngology, University of Pittsburgh School of Medicine. Each patient had BPV diagnosed by a rapid Dix-Hallpike maneuver that produced a torsional-upbeat nystagmus observed with infrared video goggles (see below). Prior to treatment with a heels-over-head PRM, each patient signed an informed consent form approved by the Institutional Review Board of the University of Pittsburgh. Each patient was given a demonstration of the new treatment, ie, heels-over-head rotation, and was advised, in detail, about conventional treatments available for BPV, including the Epley procedure.

The clinical characteristics of the reported patients are given in Table 1. The causes of BPV varied across the population, although the most common cause was idiopathic. Vestibular laboratory testing was available for 7 of the patients. These tests did not show any uniform pattern of abnormalities (Table 1). None of the patients had a unilateral reduced vestibular response — a finding that has been reported by others to occur with a frequency of about 40% in patients with BPV.[8]

The particle repositioning device was designed to restrain patients securely with the affected posterior semicircular canal aligned with the sagittal body plane. This was accomplished by turning each patient's head about 45° to the right or left as appropriate and then securing the head with a cloth restraint across the forehead. A photograph of the PRM device is shown in Fig 1. The device allowed 360° rotation of the patient in the sagittal body plane. Over-the-shoulder and lap belts were used for restraint. A braking mechanism could be released or engaged by the operator such that the patient could be stopped in any position throughout the rotation.

During particle repositioning, infrared video goggles (Jedmed, St Louis, Mo) were used to monitor each patient's eye movements. A video multiplexing device enabled simultaneous videotaping of both eyes, the repositioning device, and body position, so that these angular images could be reviewed at a later date. Figure 2 illustrates the multiplexed video monitor scheme used to review each patient's eye movements.

The protocol for particle repositioning using the heels-over-head rotation device consisted of an initial backward pitch rotation by about 115°. This portion of the protocol was essentially identical with a Dix-Hallpike maneuver, except that 1) the patient was brought into position more rapidly than at the bedside, 2) the patient's head was pitched backward by a larger angle than typically used in a Dix-Hallpike maneuver, and 3) visual fixation was completely absent because of the presence of infrared goggles. The patient was held in this supine head-down position until his or her nystagmus and vertigo ceased. This time ranged between 30 and 133 seconds, with an average of 68 ± 31 seconds. Then, patients were rotated further in the sagittal plane until they reached about 220°, which corresponds to a prone head-down position (Fig 1C). Most (10 of 11) patients were stopped in this position for a duration that depended upon their signs and symptoms. If nystagmus was noted, patients were maintained in the prone head-down position until the nystagmus disappeared. Otherwise, patients were maintained between 5 and 31 seconds in the prone head-down position and then brought back into the upright position by continuing the heels-over-head rotation. Although rotational velocity was not measured explicitly, each rotational motion — that is, rotation from upright to pitch back-

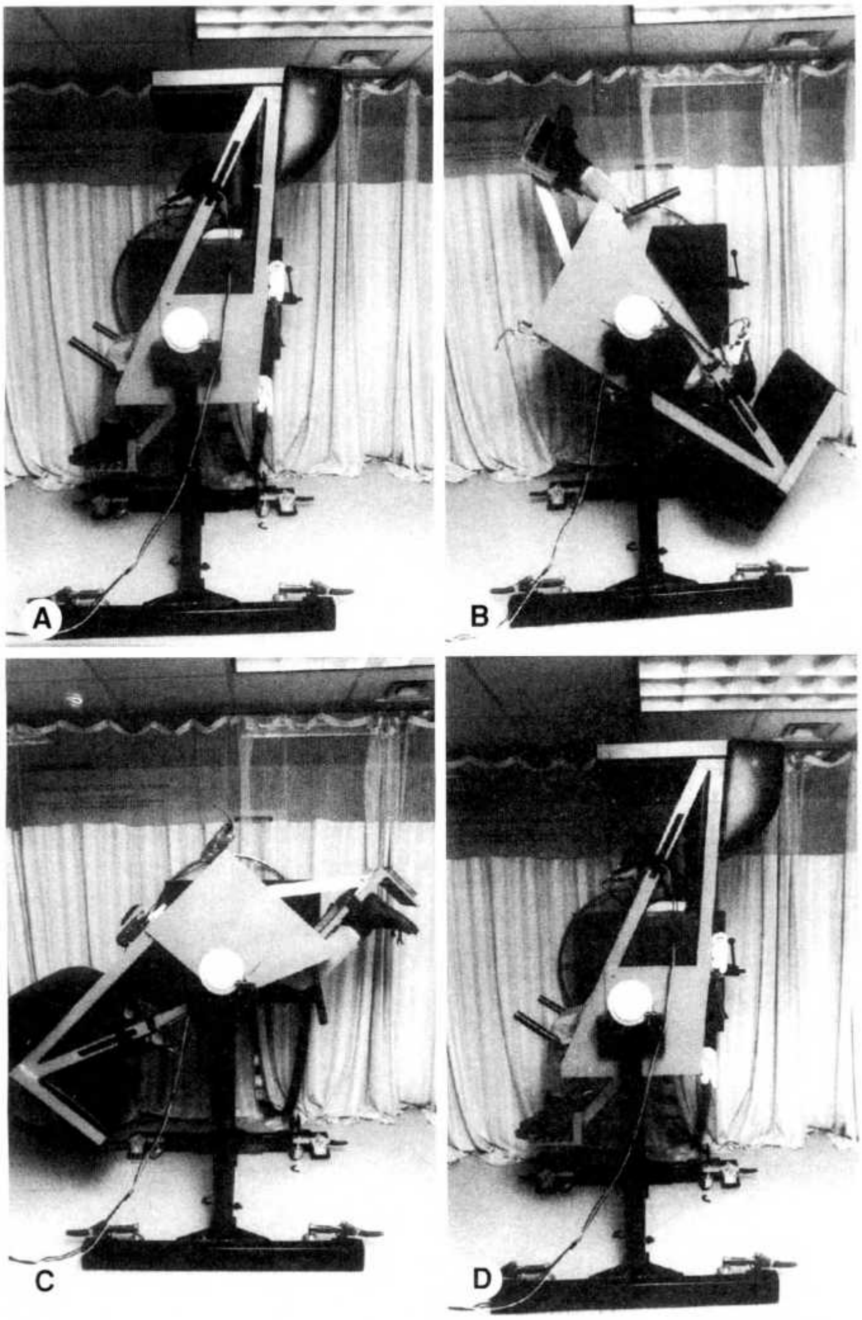

Fig 1. Photographs of heels-over-head rotational device. A) Upright position prior to particle repositioning. B) Supine head-down position equal to about 115° of backward pitch. C) Prone head-down position equal to about 220° of backward pitch. D) Upright position following particle repositioning.

ward, rotation from pitch backward to prone head-down, and rotation from prone head-down to upright — was accomplished at a rotational velocity of approximately 100°/s. The patient who was not stopped in the prone head-down position also was rotated at a velocity of about 100°/s. During particle repositioning, skull vibration was used in 7 of the 11 patients on an unselected basis.[9] Vibration was effected by placing a pocket-sized vibrator (Medical-Surgical Innovations, West Palm Beach, Fla) that generated a vibration at 60 Hz against the skull, just behind the affected ear. The vibratory device was held in place by the cloth strap that secured the infrared video goggles.

Following the initial heels-over-head particle repositioning, a second repositioning was completed for all but 1 patient, in whom the repeat repositioning was aborted. (The number of repositionings is given in Table 2.) Two patients underwent a third repositioning, and 1 of these patients underwent 2 additional repositionings. The decision to perform 1 or more additional PRMs was based on a judgment by the physician regarding the presence of nystagmus during the preceding PRM and on the patient's ability to tolerate the procedure. Patients were asked to remain upright for either 24 hours (7 patients) or 48 hours (4 patients), depending on the preference of the physician performing the PRM.

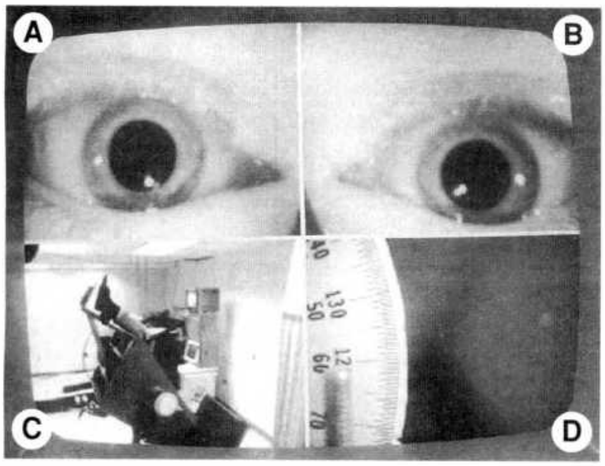

Fig 2. Photograph of split-screen video monitor used to review each patient's eye movements induced by Dix-Hallpike maneuvers and particle repositioning. A) Right eye. B) Left eye. C) View of heels-over-head repositioning device. D) Vernier of chair pitch.

RESULTS

In general, heels-over-head particle repositioning was very well tolerated, even in those patients who required multiple maneuvers. Despite the fact that being inverted is not a natural position, our patients did not complain of discomfort. We surmise that the patients' comfort was closely related to the use of highly secure restraints, which minimized motion of the patient with respect to the repositioning device during heels-over-head rotation. Many of our patients expressed their willingness to undergo a heels-over-head maneuver again if necessary and stated that they would recommend the procedure for other individuals suffering from BPV.

TABLE 2. NYSTAGMUS DIRECTION DURING INITIAL HEELS-OVER-HEAD PARTICLE REPOSITIONING MANEUVER AND NUMBER OF REPEAT REPOSITIONINGS PERFORMED

Patient No.	*Side of Lesion*	*Prone Head-Down (220°) Position*	*Upright (0°) Position*	*No. of Repeat Repositionings Performed*
1	R	Downbeat	Right beating	1
2	R	Torsional upbeat	Downbeat	1
3	L	None	None	1
4	L	Torsional upbeat	Left beating, then downbeat	0
5	L	None	None	1
6	R	None	None	2
7	R	None	None	1
8	R	Not available	None	1
9	L	None	None	1
10	R	Upbeat	None	1
11	L	None	Downbeat, then left beating	4

All 11 patients reported herein experienced an immediate resolution of their BPV following heels-over-head rotation (Table 3). All but 1 of the patients were symptom-free when contacted by telephone 4 to 271 days following treatment. One patient was lost to follow-up beyond 4 days because of a subsequent illness. The 1 patient who experienced a recurrence of symptoms, at 4 weeks, was the individual whose repeat repositioning was aborted.

As per the inclusion criteria for this study, eye movements recorded following the initial 115° backward pitch, ie, the Dix-Hallpike maneuver, demonstrated a torsional-upbeat nystagmus in which the upper pole of the eyes beat toward the affected ear, ie, typical benign paroxysmal positional nystagmus. We observed either a very brief latency or no latency between the positioning and the onset of nystagmus in each of our patients (Table 4). The duration of this initial nystagmus ranged from 12 to 29 seconds, with an average of 19 ± 6 seconds. Five patients developed a reversal phase of their nystagmus while still in the Dix-Hallpike position. In each case, the direction of this nystagmus was downbeat, and it was short-lived.

A detailed analysis of the eye movements that occurred *during* the PRM indicated that in the prone head-down position, 2 patients exhibited torsional-upbeat nystagmus, 1 patient exhibited downbeat nystagmus, 1 patient exhibited upbeat nystagmus, and 6 patients had no nystagmus (Table 2). One patient was not stopped in the prone head-down position. Upon return to the upright position, 3 patients exhibited nystagmus with a downbeat component, 3 patients exhibited nystagmus with a horizontal component, and 7 patients exhibited no nystagmus. Two

TABLE 3. OUTCOME OF HEELS-OVER-HEAD MANEUVER

Patient No.	*Immediate Resolution of Symptoms?*	*Recurrence of Symptoms?*	*Length of Follow-up (d)*
1	Yes	No	131
2	Yes	No	32
3	Yes	No	68
4	Yes	Yes, 1 mo	30
5	Yes	No	271
6	Yes	No	4*
7	Yes	No	259
8	Yes	No	115
9	Yes	No	195
10	Yes	No	125
11	Yes	No	76

*This patient's length of follow-up was limited by occurrence of subsequent, unrelated disorder.

TABLE 4. NYSTAGMUS IN SUPINE HEAD-DOWN POSITION FOLLOWING DIX-HALLPIKE MANEUVER

Patient No.	*Latency (s)*	*Duration of Initial Nystagmus (s)*	*Direction*	*Reversal Phase*
1	0	18	Torsional upbeat	Downbeat
2	0	27	Torsional upbeat	Downbeat
3	5	14	Torsional upbeat	Downbeat
4	0	25	Torsional upbeat	None
5	1	14	Torsional upbeat	None
6	0	21	Torsional upbeat	None
7	0	21	Torsional upbeat	None
8	0	29	Torsional upbeat	Downbeat
9	0	12	Torsional upbeat	None
10	0	13	Torsional upbeat	None
11	1	20	Torsional upbeat	Downbeat

patients had both vertical and horizontal components to their nystagmus.

During the repeat heels-over-head PRM, the backward pitch to a 115° supine head-down position produced no nystagmus in 7 of the 11 patients, typical BPV-type nystagmus in 2 of the 11 patients, and downbeat nystagmus in 2 of the 11 patients. Two of the 6 patients stopped in the prone head-down position during the repeat PRM exhibited downbeat nystagmus. None of the 10 patients who completed a repeat PRM had nystagmus upon return to upright. A repeat PRM was aborted in 1 of our 11 patients.

DISCUSSION

The results of this investigation suggest that 360° sagittal-plane heels-over-head rotation is an efficacious therapy for BPV. These results are in agreement with other reports of a similar therapeutic maneuver.[6,7] The success of such a maneuver lends further support to the canalithiasis theory of BPV, first proffered by Hall et al[2] in 1979. The clinical advantage of a PRM using a mechanical device as compared to a bedside PRM is that patients who have limitations of movement, either from obesity or from musculoskeletal disorders such as cervical spine disease, can undergo particle repositioning with comparatively little difficulty. Despite these advantages, heels-over-head sagittal plane rotation is unlikely to become a routine method of treatment for BPV, because it is cumbersome and potentially uncomfortable for the patient. Bedside PRM will probably continue to be the most common maneuver for treating BPV.

Are there aspects of a patient's clinical presentation or aspects of the eye movements during a heels-over-head PRM that are correlated with outcome? Our data suggest that sagittal-plane heels-over-head rotation is efficacious regardless of the cause of the BPV or the presence or absence of other vestibular laboratory abnormalities. Moreover, eye movements during and following a heels-over-head PRM do not seem to predict outcome. This result supports the generally accepted pathophysiology for BPV, ie, free-floating debris in the endolymph of the posterior semicircular canal.

Our investigation included the recording and subsequent review of eye movements during the diagnosis and treatment of BPV. These recordings allow some speculations regarding BPV and its treatment by particle repositioning. Our data indicated minimal or no latency between positioning of the patient in the supine head-down, ie, the Dix-Hallpike, position and the onset of nystagmus. This absence of a latency has been observed by others, but is notably uncommon.[10,11] We speculate that the immediate onset of nystagmus observed in all but 1 of the patients in our study, in contrast to the previous observations of others, can be attributed to 3 factors. First, patients were moved from the seated to the supine head-down position much more quickly in our heels-over-head rotation device than can be accomplished in a bedside Dix-Hallpike maneuver. This abrupt repositioning may have allowed gravity to act on the endolymphatic debris optimally, in that the portion of the canal through which the debris moves immediately following supine head-down positioning becomes more or less vertical. By comparison, during a bedside Dix-Hallpike maneuver, debris is typically being moved at an oblique angle because of the several seconds required to bring the patient's head into position and the amount of head pitch. That is, in the heels-over-head rotation device, patients are brought to approximately 115° supine head-down rapidly, whereas in the bedside Dix-Hallpike maneuvers, patients are often brought gradually only to supine.

Second, our infrared video goggles system allowed for a complete absence of visual fixation. This contrasts with Frenzel glasses, which reduce fixation significantly but do not eliminate it. Possibly, the very low-amplitude nystagmus that we observed in the earliest moments following the Dix-Hallpike maneuver in this study is typically suppressed by most patients even while wearing Frenzel glasses. This argument is particularly applicable to the vertical component of the nystagmus associated with BPV and less applicable to the torsional component, which is relatively unaffected by visual fixation.[12]

Third, our video recording system allowed us to observe an extremely low-amplitude nystagmus. Whereas observing such low-amplitude nystagmus can be quite difficult, if not impossible, while performing a Dix-Hallpike maneuver at the bedside, we could review our video recordings subsequent to the PRM.

Our observation of the immediate onset of nystagmus suggests that the criteria for a certain diagnosis of BPV should be revised. Specifically, the importance given to the presence or absence of a latency between positioning and the onset of nystagmus should take into account the speed with which a patient is repositioned, the final pitch position of the head, and the means used to observe the patient's nystagmus. With the positioning technique described in this paper, the absence of a latency between positioning and the onset of nystagmus is certainly consistent with BPV.

The nystagmus observed immediately following the initial Dix-Hallpike maneuver was uniform in direction, but varied across the population in terms of duration and the presence or absence of a secondary-phase nystagmus, ie, a reversal of the nystagmus direction. The direction of eye movements immediately *following* a Dix-Hallpike maneuver is dependent on the direction of gaze; when a patient is looking toward the lesioned ear, his or her eye movements are predominantly torsional, whereas in looking away from the affected ear, the eye movements are typically vertical.[13] Our patients were asked to keep their eyes in the primary position, that is, to gaze in the direction in which the nose was pointing. Review of our patients' eye movements indicated that each patient was indeed gazing more or less in the primary position, and each was observed to have a torsional-upbeat nystagmus typical of BPV.

The duration of nystagmus following the initial Dix-Hallpike maneuver was quite variable. We speculate that those patients with a long duration of nystagmus in the Dix-Hallpike position represent individuals who had a large amount of debris in their endolymphatic space that had agglomerated and was freely mobile. Another possible factor that could have influenced the duration of nystagmus following the Dix-Hallpike maneuver was the location of the debris while the patient was in the upright position, ie, *prior* to the maneuver.

A reversal of nystagmus following the initial Dix-Hallpike maneuver was seen in about half of our patients and was downbeat in each. This reversal of nystagmus might suggest a peripheral vestibular mechanism wherein the semicircular canal cupula reverses direction, either as a result of its own mechanical dynamic properties or as a result of the pressures and endolymphatic flows induced by the movement of the endolymphatic debris. Such an explanation is unlikely, because the reversal phase nystagmus had no torsional component and was uniformly downbeat. A more likely explanation for the reversal of nystagmus direction during the Dix-Hallpike maneuver is a central mechanism, possibly through the velocity storage system.[14,15] Velocity storage represents a hypothetical mechanism that prolongs the neural activity in the central nervous system induced by peripheral vestibular stimulation. Velocity storage is strongest for horizontal movements and essentially absent for torsional movements.[16] For vertical eye movements, velocity storage is present, though weak.[17] Thus, the reversal of nystagmus following the Dix-Hallpike maneuver may have contained only a vertical component, because torsional velocity storage is essentially nonexistent; that is, only the vertical component of the nystagmus induced by the Dix-Hallpike maneuver could be "stored" and then reverse direction.

We noted that those patients with a reversal phase of their nystagmus following the Dix-Hallpike maneuver tended to have a longer average duration of the initial, ie, torsional-upbeat, phase of nystagmus than those patients without a reversal phase: 24 ± 5 seconds versus 17 ± 5 seconds (Table 4). We speculate that those of our patients whose nystagmus reversed during Dix-Hallpike maneuvers had a larger quantity of mobile debris than those patients who did not have a reversal phase of nystagmus. Our speculation is based on the well-known finding that during constant-velocity rotational testing, a reversal of nystagmus is particularly dependent on the magnitude of the vestibular stimulation. For example, a reversal phase nystagmus is seen only when the stimulus amplitude exceeds about 100°/s.[18]

Only 4 of our patients had nystagmus in the prone head-down position, which was of variable direction. Movement of debris away from the posterior semicircular canal ampulla and toward the vestibule would be expected to produce torsional-upbeat nystagmus similar to that observed in the Dix-Hallpike maneuver. This was described by Parnes and Price-Jones[4] in 1993. However, *downbeat* nystagmus has been observed in both humans and nonhuman primates when they are prone. Thus, the variability in direction of nystagmus observed when our patients were placed in the prone head-down position during heels-over-head particle repositioning may reflect competing influences of movement of endolymphatic debris and downbeat nystagmus induced by static positioning.

Those patients who had nystagmus during the PRM had no difference in outcome from those who did not. Thus, the suggestion by Parnes and Price-Jones[4] that those patients who exhibit typical benign paroxysmal positional nystagmus *during* PRM are more likely to have a favorable outcome from PRM was not supported by our study. However, using a heels-over-head device to place patients in the Dix-Hallpike position may have allowed our patients' endolymphatic debris to be moved so far from the ampulla that few of them had additional nystagmus during the remainder of the PRM. Although the presence of eye movements *during* heels-over-head particle repositioning does not appear to be critical, such eye movements may be critical when performing an Epley-type procedure, wherein debris may be closer to the cupula after the initial Dix-Hallpike maneuver than when using a heels-over-head maneuver.

When patients were brought back into the upright position at the end of their initial heels-over-head particle repositioning, 4 of the patients developed nystagmus. The direction of this nystagmus was variable. We speculate that this nystagmus was produced by movement of debris into the vestibule, where it settled onto the utricular macula and caused utricular stereocilia deflection and thus otolithic afferent stimulation. Because of the array of polarization vectors that characterize utricular afferents, the direction of nystagmus induced by such a purported utricular stimulation would depend on exactly where the debris struck the macula. For example, 1 of the patients had a nystagmus following return to upright that changed from a vertical nystagmus to a horizontal nystagmus. This suggests the possibility of movement of endolymphatic debris across the utricular macula. The 3 patients with horizontal nystagmus following PRM had the quick component of their induced nystagmus ipsilateral to the side of their lesion. This suggests that the repositioned endolymphatic debris may have settled into the medial aspect of the utricular macula. Another possible explanation for the ipsilaterally directed horizontal nystagmus seen when patients were brought back to the upright position is movement of repositioned debris into the nonampullated end of the horizontal semicircular canal. In favor of such an explanation is the reported complication, following a PRM using an Epley-type procedure, of horizontal semicircular canal BPV.[19]

Our practice of repeating the PRM until the Dix-Hallpike maneuver was negative is consistent with recommendations in the literature[11,20] and is based on the rationale that the initial PRM might not have moved all of the debris into the vestibule. Any debris that had not been repositioned during the initial maneuver could be repositioned during the repeat maneuvers. Although the pitch backward at the beginning of a repeat positioning could cause endolymphatic debris to reflux back into the posterior semicircular canal endolymph, such free-floating debris was considered to be quite mobile and, thus, capable of being easily repositioned again. Those patients with downbeat nystagmus following the repeat Dix-Hallpike maneuver were thought to have reflux of debris from the vestibule back into the endolymph of the posterior semicircular canal. Thus, we recommend that if a Dix-Hallpike maneuver is performed in a patient with BPV even following a PRM, that a repeat PRM should be completed so that any endolymphatic debris that may have refluxed into the posterior semicircular canal can be repositioned back into the vestibule.

Several unresolved issues regarding the treatment of BPV using a PRM remain, including 1) the need for patients to remain upright for 1 or 2 days following a PRM, 2) the need to repeat the Dix-Hallpike maneuver following the initial PRM to confirm the success of the maneuver, and 3) the need for using vibration of the skull during particle repositioning.[9]

Although our study did not specifically address these unresolved issues, our data provide information regarding some of them. Vibration of the skull was used in only 7 of our 11 cases, and there was no apparent influence on outcome. Four of the 11 patients were instructed to remain upright for 48 hours; 7 of the 11 patients were instructed to remain upright for 24 hours. This aspect of treatment also appeared to have no effect on outcome. Thus, the issues of skull vibration and the need to remain upright following PRM remain unresolved. However, on the basis of findings in this study and results in the literature, our current clinical practice includes 1) some form of skull vibration to aid in the process of repositioning debris through the endolymph, since vibration has no adverse effects and might be beneficial, 2) a recommendation that patients remain upright for at least 1 day, since debris appears to be free-floating in some patients following a PRM and thus may reflux back into the posterior semicircular canal, and 3) repeated particle repositioning until the Dix-Hallpike maneuver is negative, since a single maneuver may not be entirely effective.

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NEUROTOLOGICAL AND AUDITORY BRAIN STEM RESPONSE FINDINGS IN HUMAN IMMUNODEFICIENCY VIRUS–POSITIVE PATIENTS WITHOUT NEUROLOGIC MANIFESTATIONS

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NICOLETTA BARONI, MD EUGENIO PALLESTRINI, MD

GENOA, ITALY

Neurologic manifestations of human immunodeficiency virus (HIV) infection, rather than being a late complication of the disease, are principally correlated with the early central nervous system (CNS) localization of HIV. The CNS may be infected in the early stages of acquired immunodeficiency syndrome (AIDS) without evidence of neurologic disorders. Evoked potentials (visual, auditory, and somatosensory) and electronystagmographic test batteries have proven to be very sensitive in showing subclinical CNS disorders due to HIV. In this study, auditory brain stem response (ABR) and electronystagmographic test battery findings (smooth pursuit, saccades, caloric test) were performed in 29 neurologically asymptomatic, HIV-positive subjects at different stages of the disease. Compared to results of a control group, the ABR latencies of waves V, I, and III and interpeaks I-V and III-V were significantly increased in HIV patients. The same parameters did not differ significantly among the stages of the disease. In HIV-positive subjects, the accuracy of saccades was significantly reduced, while latency was normal. The velocity and the gain of pursuit were significantly reduced in HIV-positive patients, and 15 of 29 patients showed corrective saccades. Caloric tests revealed qualitative nystagmus abnormalities in 82% of HIV patients, while quantitative parameters were normal. The present results confirm that CNS involvement by HIV occurs early in the course of the disease. In particular, HIV does not seem to affect the labyrinth or the eighth cranial nerve, as demonstrated by the normality of the I-III value of the ABR and of the quantitative parameters of the caloric responses, but it does appear to involve the brain stem acoustic pathways, pontocerebellar pathways, and supratentorial areas.

KEY WORDS — auditory brain stem response, caloric tests, human immunodeficiency virus, pursuit, saccades.

INTRODUCTION

Neurologic manifestations of human immunodeficiency virus (HIV) infection, rather than being a late complication of the disease (such as opportunistic infections, Kaposi's sarcoma, and lymphoma), are principally correlated with the early central nervous system (CNS) localization of HIV.[1]

The most often documented neurologic complication of HIV disease is subacute encephalitis, and numerous studies[2-4] have implicated HIV as the etiologic agent responsible for this condition. Moreover, the isolation of HIV in the CNS, peripheral nerves, and cerebrospinal fluid (CSF), both in patients affected by subacute encephalitis and in patients without neurologic symptoms,[2,5,6] strongly suggests that the CNS is subject to infection early in the course of HIV disease.

From the clinical standpoint, many examinations, ie, neurologic examination, psychological test batteries,[7] CSF studies, magnetic resonance imaging (MRI), and computed tomography (CT),[8] have failed to determine subclinical neurologic lesions. Evoked potentials and neurotological test batteries, on the other hand, have proven successful in the study of these disorders.

Evoked potentials have been used by numerous authors.[3,4,9-16] Auditory brain stem response (ABR) findings in HIV-positive patients without neurologic signs reveal the progressive involvement of acoustic pathways due to viral infection; significant delay of the latencies of peaks V, III, and I and of I-V and III-V interwave values in HIV subjects compared to controls have been recorded. These results suggest an HIV action on the upper brain stem.[10] The progressive delay of peak I found in infected patients has been interpreted as a cochlear disorder due to HIV.[14] The alteration of cognitive and visual evoked potentials seems to be correlated to early involvement of the cerebral cortex in these patients.[4,12]

Neurotological studies in HIV patients[17-21] have documented abnormalities in up to 34% of asymptomatic seropositive subjects. Prospective studies[20] show that such alterations are progressive. Moreover, one study of a group of 29 HIV-positive subjects[16] found saccade velocity impairment and gain diminution of smooth pursuit.

The purpose of the present study was, specifically, to verify through ABR the brain stem and cochlear

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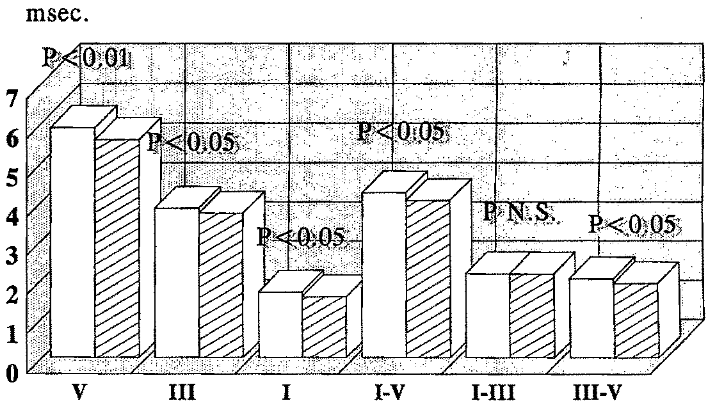
From the Otorhinolaryngology Division, Head and Neck Department, San Martino Hospital, Genoa, Italy.

CORRESPONDENCE — Eolo Castello, MD, ENT Division, Head and Neck Department, San Martino Hospital, Largo Benzi 10, 16100 Genova, Italy.



	V	III	I	I-V	I-III	III-V
HIV POSITIVES	**5.862**	**3.815**	**1.668**	**4.217**	**2.138**	**2.011**
NORMALS	**5.559**	**3.686**	**1.553**	**4.015**	**2.132**	**1.892**

☐HIV POSITIVES ▨NORMALS

Fig 1. Mean values and t-test results of auditory brain stem response (ABR) peak latency and interwave values (roman numerals) in subjects who are positive for human immunodeficiency virus (HIV) and in normal subjects. N.S. — not significant.

involvement in a group of HIV-positive subjects without neurologic symptoms at 3 different stages of the disease and, in general, to test the sensitivity of a neurotological protocol.

MATERIALS AND METHODS

Twenty-nine HIV-positive subjects (18 men, 11 women ages 20 to 41 years), neurologically asymptomatic and with bilateral normal hearing thresholds (less than 25 dB normal hearing level [nHL]), were enrolled in this study. None had a history of alcohol consumption. Classified according to Centers for Disease Control (CDC) criteria (stage II, asymptomatic patients; stage III, patients with persistent generalized lymphadenopathy; and stage IV, patients with additional complications of HIV infection), there were 8 patients at CDC stage II, 10 at CDC stage III, and 11 at CDC stage IV.

The control group included 20 HIV-negative subjects (10 men, 10 women) with bilateral normal hearing (20 dB nHL or better at all frequencies) and without signs or history of neurologic disease.

Auditory Brain Stem Response. A Nicolet C4 evoked potentials analyzer was used. The stimuli were 2,000 clicks (duration 0.1 millisecond; alternating polarity; rate 10 stimuli per second) delivered at 90 dB hearing level (HL) through TDH headphones. Surface silver chloride electrodes were placed on the forehead, on the mastoid, and on the vertex. The biologic signal was filtered at a setting of 100 to 3,000 Hz. The absolute values of waves I, III, and V and the interpeak latencies of I-V, I-III, and III-V were evaluated. The binaural mean of the values of these parameters of the HIV group was compared with that of the control group by analysis of variance (ANOVA). Scheffé variance analysis was used to determine significant differences of ABR results among the stages of HIV disease.

Neurotological Tests. Electronystagmographic testing included the study of oculomotor reflexes (smooth pursuit and saccades) and vestibulo-ocular reflex (VOR) with caloric tests (bithermal 30°C and 44°C stimulation according to Hallpike-Fitzgerald).

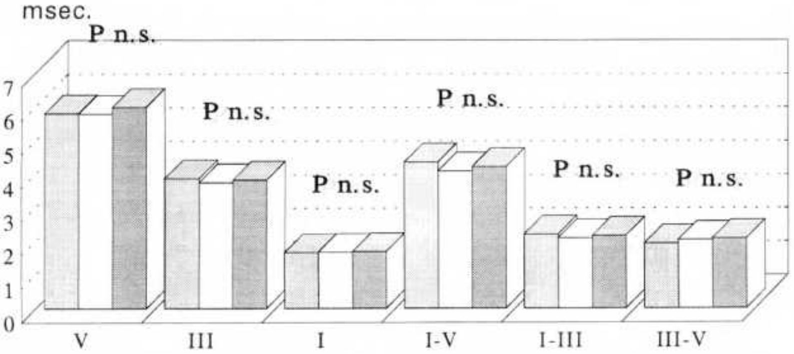
A Nicolet Nystar computerized electronystagmography system was used for this purpose. The latency and accuracy of fixed and random saccades were analyzed. Smooth pursuit was studied at 0.4 and 0.2 Hz, and gain and amplitude were calculated. For the caloric tests, nystagmus, maximum slow component velocity (MSCV), directional preponderance, labyrinthine paresis, and qualitative anomalies (dysrhythmia, square waves) were evaluated.

Neurotological evaluation was comprehensive of spontaneous nystagmus (with and without ocular fixation) and of positional nystagmus.

RESULTS

Auditory Brain Stem Response. The ABR data of both ears were pooled in order to calculate mean binaural values. The ABR recordings were morphologically altered (wave amplitude diminution, lack of peak identification) in 18 HIV-positive patients (57.7%). In particular, peak V could be identified in all cases in both ears, while peak III was absent in 1 left ear (3.4%) and in 1 right ear (3.4%), and peak I was undetected in 1 left ear (3.4%) and in 3 right ears (10.4%).

Figure 1 reports the comparative ABR results of HIV-positive subjects and controls and the results



STAGE II	**5.796**	**3.875**	**1.654**	**4.355**	**2.197**	**1.921**
STAGE III	**5.781**	**3.751**	**1.667**	**4.089**	**2.079**	**2.029**
STAGE IV	**5.985**	**3.832**	**1.681**	**4.222**	**2.151**	**2.071**

Fig 2. Statistical comparison (Scheffé's variance analysis) of ABR results among disease stages. n.s. — not significant.

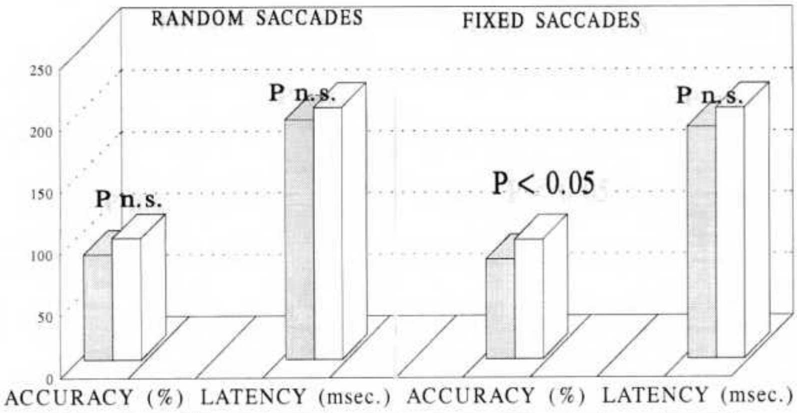
of ANOVA analysis. The latencies of waves V, III, and I and the interpeak values of I-V and III-V increased significantly (as was statistically confirmed) in HIV-positive patients, while I-III interpeak values did not differ from those of the control group.

Figure 2 shows the comparative ABR results in the different stages of HIV infection. Statistical analysis (Scheffé's variance analysis) revealed no significant variations among the different stages of the disease.

Neurotological Tests. None of the HIV-positive patients showed spontaneous or positional nystagmus. Figure 3 shows the comparative results of saccades of the HIV-positive group and of the control group. Saccade alterations were found in 15 of the 29 HIV-positive subjects. In particular, statistical analysis showed a significant decrease of the accuracy of fixed saccades, while latency values were normal. Random saccades were also normal. Figure 4 shows a case of saccade hypometria with normal latency values.

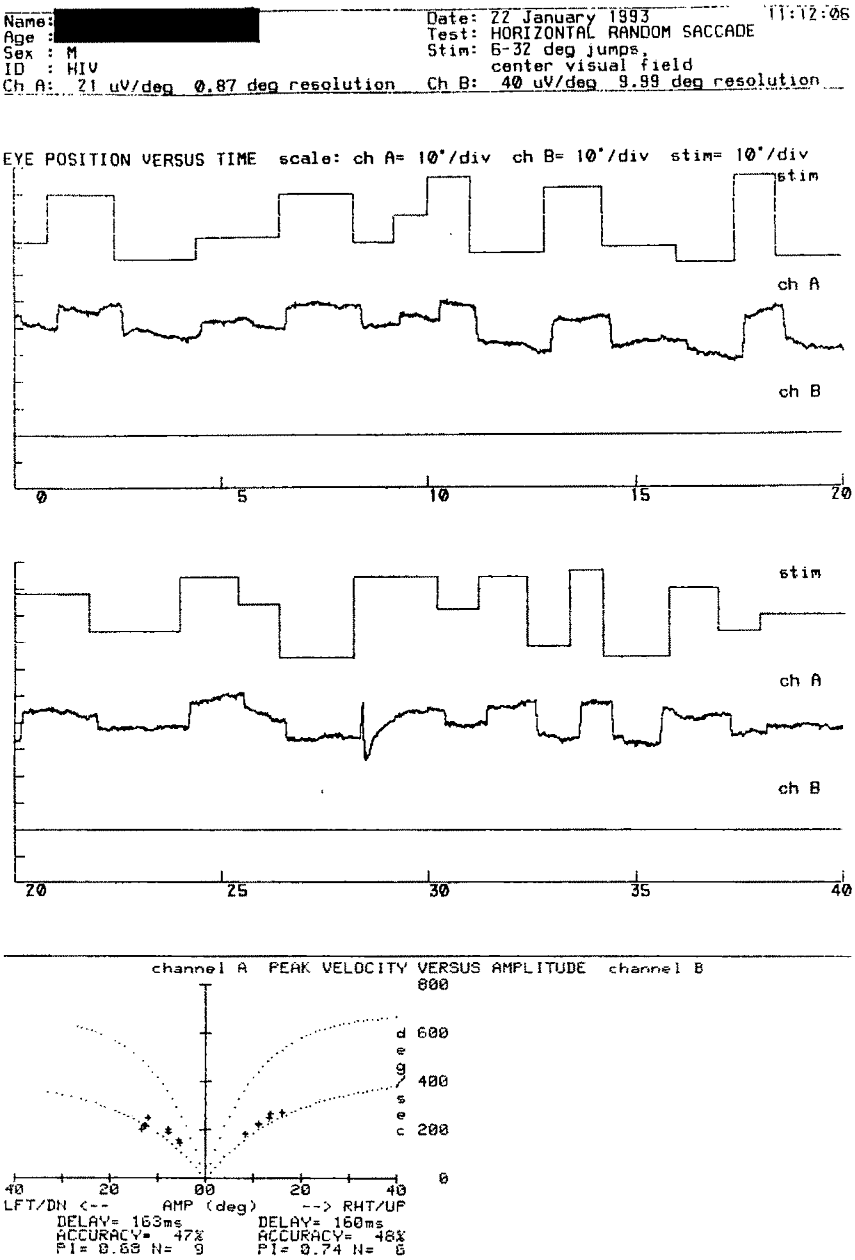
Figure 5 shows comparative results and statistical analysis of smooth pursuit of HIV-positive and normal subjects. We found a significant diminution of 0.2-Hz pursuit velocity and a significant decrease of the gain of 0.4-Hz pursuit. Fifteen of the 29 subjects showed corrective saccades. Figure 6 shows 0.2-Hz horizontal pursuit modified by corrective saccades (cogwheel pursuit).

Caloric tests showed, from the quantitative standpoint, no significant variations of nystagmic reaction parameters in HIV-positive patients (see Table). Qualitative nystagmic alterations were observed in 24 subjects (82%). In particular, square waves were present in 32% of subjects, microbeats in 28%, and dysrhythmia in 63%. Figure 7 shows nystagmus dys-



HIV POSITIVE	**85.087**			**194.29**			**80.83**		**187.91**
NORMALS	**98.3**			**204.29**			**96.6**		**203.5**

Fig 3. Mean values and t-test results of saccades in HIV-positive and normal subjects.



```
Name: [redacted]
Age :
Sex : M                                    Date: 22 January 1993              11:12:06
ID  : HIV                                  Test: HORIZONTAL RANDOM SACCADE
                                           Stim: 6-32 deg jumps,
                                                 center visual field
Ch A:  21 uV/deg  0.87 deg resolution      Ch B:  40 uV/deg  9.99 deg resolution
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DELAY= 163ms           DELAY= 160ms
ACCURACY=  47%         ACCURACY=  48%
PI= 0.68 N=  9         PI= 0.74 N=  6
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Fig 4. Hypometric saccades in HIV patients (accuracy less than 50% on both sides).

rhythmia with square waves. Quantitative nystagmic parameters were normal.

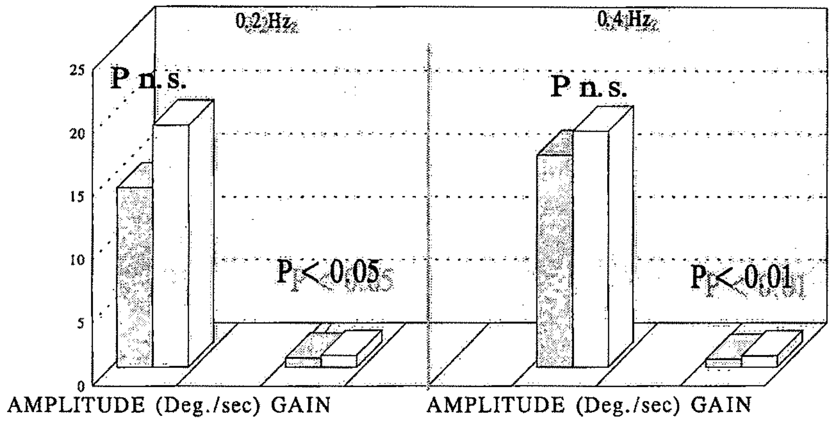
DISCUSSION

The isolation of HIV in the CNS, peripheral nerves, and CSF in patients without neurologic symptoms[2,5,6] suggests that the CNS is subject to infection early in the course of HIV disease. Because classic imaging techniques, such as MRI and CT, and clinical examination are not always reliable in determining subclinical neurologic lesions, evoked potentials and neurotological test batteries could prove useful for the study of early CNS involvement by HIV.

Our results, obtained through ABR and neurotological testing, indicate multiple asymptomatic dysfunctions of the CNS due to HIV, regardless of the stage of the disease.

The ABR testing results show an increase of the latency of all main components and of I-V and III-V interwave values — findings that clearly express dysfunction of upper brain stem acoustic pathways. In particular, while the brain stem certainly seems to be subject to HIV infection, the acoustic nerve appears not to be significantly affected, as demonstrated by the normality of I-III interwave values. Our findings are in keeping with those of other authors.[22,23]

The alterations of smooth pursuit observed in the HIV patients in our study reveal nonspecific CNS



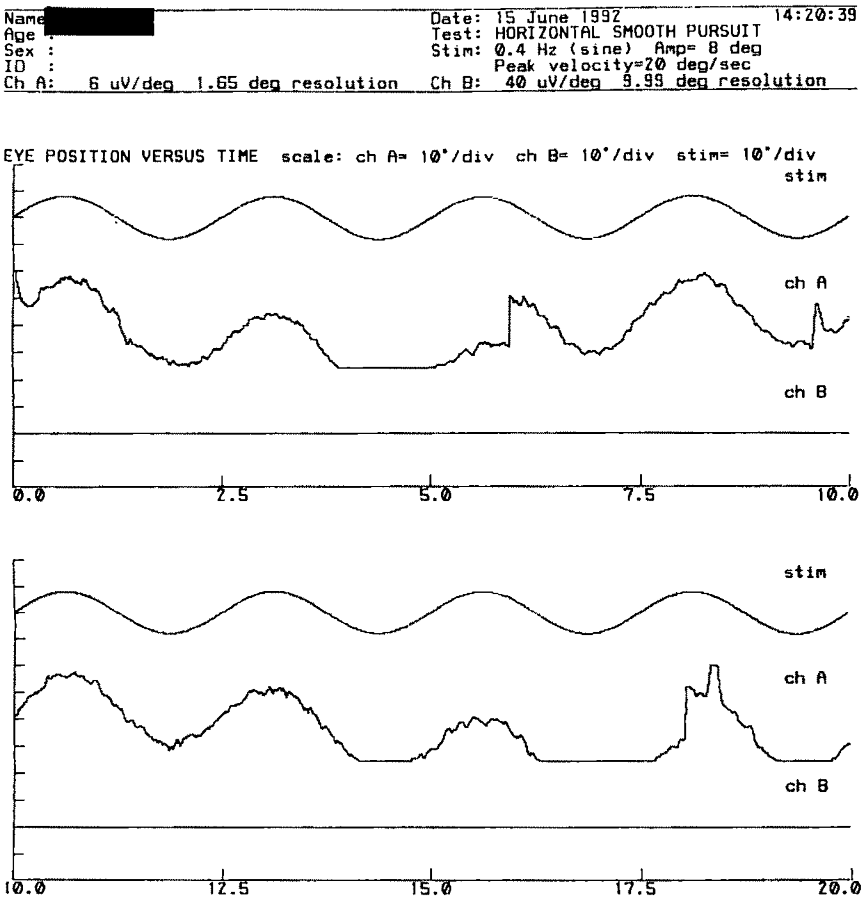
HIV POSITIVE	**14.2**		**0.723**			**16.82**		**0.621**
NORMALS	**19.17**		**0.921**			**18.74**		**0.88**

▨HIV POSITIVE ☐NORMALS

Fig 5. Mean values and t-test results of smooth pursuit parameters in HIV-positive and normal subjects.

disorders. In particular, gain and amplitude diminution and the presence of corrective saccades could very well mark the involvement of pons-cerebellar pathways and supratentorial areas. Saccade findings in our patients, particularly hypometria, might imply HIV-induced damage to pretectal and paramedial pontine regions, which are fundamental for the generation and modulation of saccades. In this regard, our results disagree with those of Rosenhall et al,[16] who found velocity diminution of saccades without alterations of accuracy in HIV subjects.

The normality of caloric test findings in our group of patients does not exclude, at least from a quantitative standpoint, labyrinthine involvement by HIV in the early stages of the disease. On the contrary,



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Name [redacted]                             Date: 15 June 1992                14:20:39
Age  :                                      Test: HORIZONTAL SMOOTH PURSUIT
Sex  :                                      Stim: 0.4 Hz (sine)  Amp= 8 deg
ID   :                                            Peak velocity=20 deg/sec
Ch A:   6 uV/deg  1.65 deg resolution       Ch B:  40 uV/deg  9.99 deg resolution

EYE POSITION VERSUS TIME   scale: ch A= 10°/div   ch B= 10°/div   stim= 10°/div
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Fig 6. Cogwheel-shape horizontal smooth pursuit in HIV patient.

QUANTITATIVE PARAMETERS OF CALORIC TESTS IN HIV-POSITIVE AND NORMAL SUBJECTS

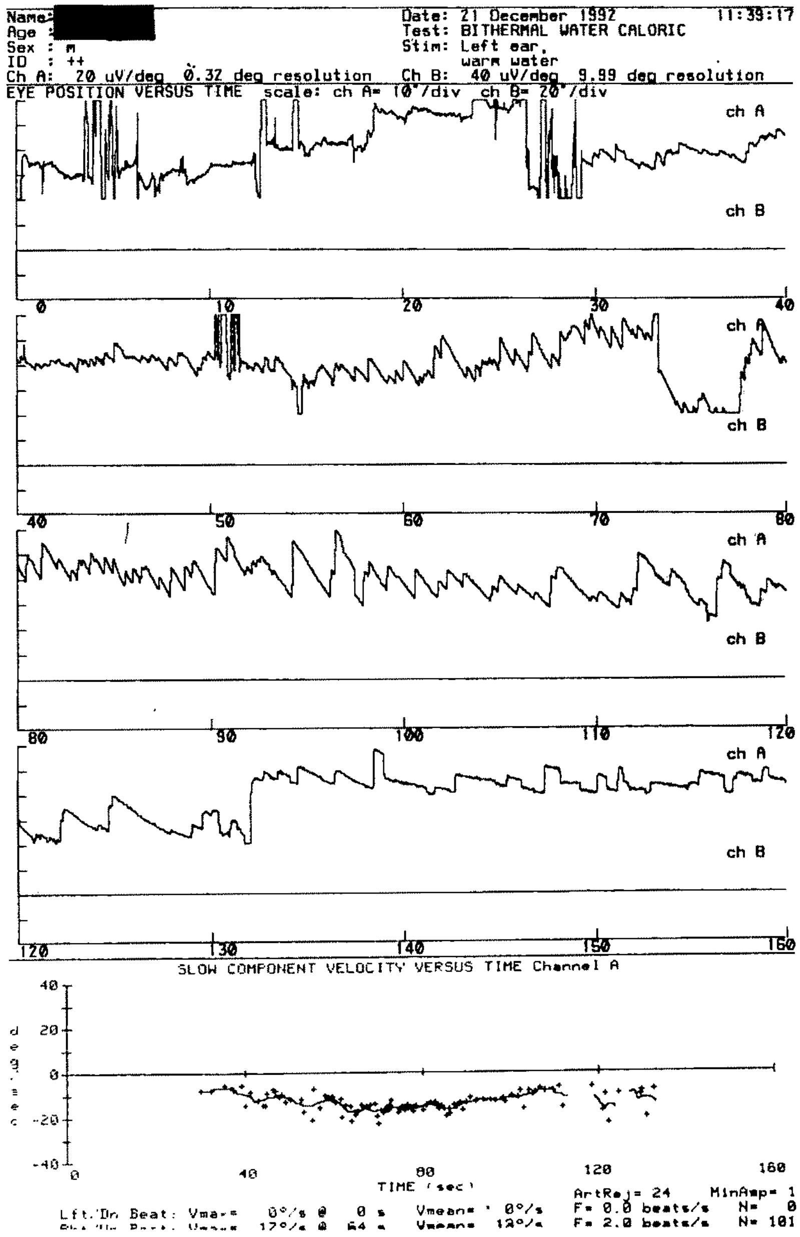
	Side	*HIV*	*Controls*	*p*
MSCV 44°C	R	28.32 ± 15.6	31.92 ± 12.6	NS
MSCV 44°C	L	23.65 ± 11.8	29.88 ± 11.9	NS
MSCV 30°C	R	20.83 ± 14.07	22.65 ± 12.06	NS
MSCV 30°C	L	22.40 ± 12.38	25.01 ± 14.1	NS
Beats 44°C	R	122.58 ± 63.1	123.84 ± 44	NS
Beats 44°C	L	121.65 ± 60.8	125.44 ± 53	NS
Beats 30°C	R	127.11 ± 67.9	119.87 ± 44	NS
Beats 30°C	L	112.94 ± 83.59	120.12 ± 53	NS

Data are means ± SD; p is for *t*-test.

HIV — human immunodeficiency virus, MSCV — maximum slow component velocity, NS — not significant.

the qualitative nystagmic alterations we observed, eg, square waves, do seem to indicate involvement of cerebellum-vestibular pathways.

Overall, our findings uphold previous results showing neurotropism of HIV and a direct action of the virus on the CNS, even in neurologically asymptomatic patients. The ABR and neurotological protocols are very sensitive tools for detecting asymptomatic CNS disorders in the early stages of HIV infection. Because neurologic disorders are manifested only in the later stages of HIV disease, these testing methods could readily be utilized in HIV-positive patients to detect neurologic complications very early in the course of infection.



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Name:[redacted]                              Date: 21 December 1992          11:39:17
Age :                                        Test: BITHERMAL WATER CALORIC
Sex : m                                      Stim: Left ear,
ID  : ++                                           warm water
Ch A:  20 uV/deg  0.32 deg resolution        Ch B:  40 uV/deg  9.99 deg resolution
EYE POSITION VERSUS TIME  scale: ch A= 10°/div  ch B= 20°/div
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                                                            ArtRej= 24      MinAmp= 1
Lft/Dn Beat: Vmax=   0°/s @   0 s   Vmean=   0°/s   F= 0.0 beats/s   N=   0
Rht/Up Beat: Vmax=  17°/s @  64 s   Vmean=  12°/s   F= 2.0 beats/s   N= 101
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Fig 7. Case of nystagmus dysrhythmia with square waves.

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SPEECH AND VOICE CHARACTERISTICS AFTER NEAR-TOTAL LARYNGECTOMY

A PRELIMINARY PROSPECTIVE STUDY

OLLIVIER LACCOURREYE, MD LISE CREVIER-BUCHMAN, MD LUCA MUSCATELLO, MD
STÉPHANE HANS, MD MADELEINE MÉNARD, MD DANIEL BRASNU, MD
PARIS, FRANCE

Selected characteristics were prospectively compared in the speech and voice of 10 patients managed with near-total laryngectomy. Tape-recorded speech samples were measured for durational features with a stopwatch. Acoustic features were analyzed with the Computerized Speech Lab and the Multidimensional Voice Program from Kay Elemetrics. Speech and voice parameters recorded preoperatively were compared with the parameters recorded postoperatively. Our data corroborate the prior reported good quality of the voice and speech after near-total laryngectomy.

KEY WORDS — near-total laryngectomy, speech and voice characteristics.

INTRODUCTION

Pearson's near-total laryngectomy was initially advocated for patients with extended glottic carcinoma, supraglottic carcinoma, and pyriform sinus carcinoma.[1,2] DeSanto et al[3] also reported the utility of near-total laryngectomy for supraglottic, pharyngeal, base of tongue, and other cancers such as thyroid cancer with anterior tracheal wall invasion. Various clinical reports and pathologic studies demonstrated the oncologic suitability and the low rate of complications — namely, aspiration and stenosis of the tracheolaryngopharyngeal shunt — of near-total laryngectomy.[1-13]

Much research has been devoted to the surgical technique, the indications, and the oncologic results.[1-13] However, to our knowledge, only 1 group of investigators reported data describing acoustic voice characteristics that exist after near-total laryngectomy.[14-16] Further, no report specifically compared the speech and voice parameters attained after near-total laryngectomy with the preoperative parameters. The present prospective study was therefore designed to evaluate the modifications of selected durational and acoustic features of speech and voice in a series of 10 patients managed with a near-total laryngectomy in our department. The independent factors of age, past medical history, postoperative complications, postoperative radiotherapy, and delay from surgery were also tested for potential correlation with the postoperative durational and acoustic features of speech and voice after near-total laryngectomy.

MATERIALS AND METHODS

Ten patients managed in our department between 1995 and 1996 with Pearson's near-total laryngectomy[1,2] took part in this preliminary prospective study. Table 1 summarizes background information regarding age, sex, employment, past medical history, tumor location, and TNM staging according to the 1997 American Joint Committee on Cancer classification system.[17] Local recurrence and distant metastasis were not encountered in the present series.

The tracheolaryngopharyngeal shunt was fashioned from the whole true vocal cord, false vocal cord, paraglottic space, and interarytenoid muscle on the non–tumor-bearing side (Vicryl 3-0 suture). The myomucosal hypopharyngeal flap described by Pearson et al[1,2] was not used in the present prospective study. The pharyngeal closure was performed in a conventional T fashion (Vicryl 2-0 suture). Pharyngeal closure required the use of a sternomastoid flap in 2 patients, and a pectoralis major myocutaneous flap in 1 patient. Management of the neck included an ipsilateral modified radical neck dissection[18] taking node levels II through IV, sparing the spinal accessory nerve, in 8 patients. An ipsilateral thyroid lobectomy and recurrent lymph node chain dissection[18] was performed in 8 patients. Five surgeons managed the patients. Postoperative radiotherapy was performed in 5 patients.

Speakers were recorded under similar conditions

From the Department of Otorhinolaryngology–Head and Neck Surgery, Hôpital Läennec, Assistance Publique des Hôpitaux de Paris, University Paris V, France.

CORRESPONDENCE — Ollivier Laccourreye, MD, Dept of Otorhinolaryngology–Head and Neck Surgery, Hôpital Läennec, 42 rue de Sèvres, 75007, Paris, France.

TABLE 1. BACKGROUND INFORMATION ON NEAR-TOTAL LARYNGECTOMY SPEAKERS

Age	*TNM*	*Sex*	*Location*	*Employment*	*Past Medical History*
58	T4N0	M	Hypopharynx	Unemployed	Tonsil carcinoma and radiotherapy
74	T4N0	M	Endolarynx	Retired	Transient cerebral ischemia and high blood pressure
48	T3N3	M	Epilarynx	Unemployed	Cardiomyopathy and depression
56*		M		Factory worker	
51	T3N2	M	Hypopharynx	Architect	Chronic pancreatitis and depression
42	T4N2	F	Hypopharynx	Pharmacist	
61†	T3N0	M	Tongue base	Factory worker	Chronic obstructive pulmonary disease
61	T3N1	M	Vallecula	Unemployed	Chronic obstructive pulmonary disease
42	T4N1	M	Hypopharynx	Librarian	
66	T4N1	M	Hypopharynx	Retired	

TNM staging according to American Joint Committee on Cancer classification system.[17]

*Patient with severe aspiration after supracricoid hemilaryngopharyngectomy.

†Patient with salvage procedure after radiotherapy failure.

in a quiet room by means of a DAT Sony DTC 60FS recorder (Sony, Paris, France) and a Lem EMU 4535 microphone (Lem Communication, Igny, France). The preoperative recording was performed the week prior to completion of the near-total laryngectomy. Early and intensive postoperative speech therapy was initiated in all cases. The patients were recorded postoperatively as soon as they could phonate with efficiency. Speakers were able to phonate using the tracheolaryngopharyngeal shunt through digital occlusion of the tracheostoma. Speakers were individually tape-recorded while performing a standard order of tasks. Speech samples included maximum sustaining of the vowel /a/ (best of 2 trials), reading aloud a monosyllabic word list, and reading a standard French text equivalent to the Rainbow Passage for 2 minutes' duration. Tape-recorded speech samples were measured for durational features with a stopwatch. Acoustic parameters were analyzed with the Computerized Speech Lab and the Multidimensional Voice Program (Kay Elemetrics, Pine Brook, NJ). The durational parameters recorded were the maximum phonation time, the speech rate, and the phrase grouping. The speaker's maximum phonation time was defined as the best attempt in seconds to maximally prolong the vowel /a/ at a comfortable intensity. The speech rate was defined as the number of words read per minute. The phrase grouping was defined as the number of words per breath or air charge. Breaths were marked by listening and observing as each subject read aloud. The acoustic parameters automatically recorded were the average fundamental frequency (F0) in hertz, the F0 standard deviation (SD) in hertz, the jitter (cycle-to-cycle variation in F0) in percent, the shimmer (cycle-to-cycle variation in amplitude) in percent, and the noise-to-harmonics ratio.

Statistical analysis was performed with Programme Conversationel de Statistique pour les Sciences et le Marketing (PCSM) software (Deltasoft Company, Meynan, France). The preoperative and postoperative durational and acoustic parameters were compared by the nonparametric Wilcoxon *t*-test. The independent factors of age, past medical history, postoperative complications, postoperative radiotherapy, and delay from surgery were also tested for potential correlation with the postoperative durational and acoustic parameters. A nonparametric Mann-Whitney U test and a linear regression with Pearson correlation were used for quantitative and qualitative variables analyses, respectively. Statistical significance was at the .05 level.

RESULTS

Postoperative Course. The postoperative course was unremarkable in 7 patients, who resumed normal swallowing before the end of the second postoperative week. Temporary postoperative salivary leakage of 7 to 15 days' duration was noted in 3 patients, and required completion of a pectoralis major myocutaneous flap in 1 patient. Salivary leakage through the shunt was not encountered.

Seven patients were able to phonate by the first postoperative month. Two patients, who presented with postoperative complications, phonated by the third postoperative month. One patient had a stenosis of the shunt that was successfully managed with dilation, and phonated by the sixth postoperative month. Speech after near-total laryngectomy was subjectively judged as good in 80% of patients according to our speech pathologist (L.C.-B.).

Durational Parameters. The postoperative maximum phonation time and the phrase grouping of speakers did not statistically vary when compared with the preoperative values. The speech rate was statistically slower after near-total laryngectomy (Table 2, p = .05). Postoperatively, speakers averaged 58 words per minute slower.

In univariate analysis, the durational parameters

TABLE 2. STATISTICAL ANALYSIS OF RELATIONSHIP BETWEEN PREOPERATIVE AND POSTOPERATIVE DURATIONAL AND ACOUSTIC PARAMETERS

Speech Characteristics	*Preoperative*	*Postoperative*	*p*
Durational parameters			
Maximum phonation time (s)			
Mean	11	15	>.05
Range	4-21	2-26	
Speech rate (words per minute)			
Mean	155	97	.05
Range	83-170	88-150	
Phrase grouping (words per breath)			
Mean	19	18	>.05
Range	6-27	4-35	
Acoustic parameters			
Average fundamental frequency (Hz)			
Mean	110.7	162.7	.05
Range	85.2-206.1	119.7-421.8	
SD of fundamental frequency (Hz)			
Mean	4.9	36.8	.05
Range	1.2-11.5	1.8-179.5	
Jitter (%)			
Mean	2.29	6.82	>.05
Range	0.38-12.2	1.12-47.28	
Shimmer (%)			
Mean	8.24	19.84	.05
Range	3.97-16.45	8.74-35.93	
Noise-to-harmonics ratio			
Mean	0.16	0.66	.02
Range	0.10-0.52	0.18-0.86	

of near-total laryngectomy speakers did not significantly vary according to the following variables: age, preoperative radiotherapy, past medical history, postoperative complications, postoperative radiotherapy, and delay from surgery.

Acoustic Parameters. The average F0 and its SD displayed greater variability postoperatively. The average F0 and its SD were statistically higher postoperatively (Table 2, p = .05). The preoperative and postoperative jitter did not differ significantly. The postoperative shimmer was statistically higher (Table 2, p = .05). The postoperative shimmer was on average twice higher. The postoperative noise-to-harmonics ratio was statistically higher (Table 2, p = .02). The postoperative noise-to-harmonics ratio was 4 times higher.

In univariate analysis, the acoustic parameters of the near-total laryngectomy speakers did not significantly vary according to the following variables: age, preoperative radiotherapy, past medical history, postoperative complications, postoperative radiotherapy, and delay from surgery.

DISCUSSION

The oncologic value of Pearson's near-total laryngectomy has been reported by various authors.[1-13] The near-total laryngectomy is therefore now considered worldwide as a valuable alternative to total laryngectomy with a tracheoesophageal shunt in patients with advanced head and neck carcinoma.[1-13] This procedure was introduced at our department in 1995, and we have found that the near-total laryngectomy filled the intermediate position between the various supracricoid partial laryngectomies and total laryngectomy with tracheoesophageal puncture and voice prosthesis insertion.[13] Our preliminary functional and oncologic results paralleled the results reported in the English-language literature.[13]

Analysis of the reported series in the English-language literature suggested that more than 95% of the patients achieved physiologic phonation after near-total laryngectomy.[1-3,6-13] Suits et al,[9] in a review of the medical literature, reported that voice quality was subjectively judged by the attending surgeon or speech pathologist as good in 86% of patients (range 76% to 100%). In the present preliminary prospective study, speech was subjectively judged as good in 80% of patients, according to our speech pathologist (L.C.-B.). In 70% of patients, speech was achieved before the end of the first postoperative month. Comparison between the preoperative and postoperative durational features (maximum phonation time, speech rate, and phrase grouping) noted that the speech rate was statistically slower (58 words per minute slower) after near-total laryngectomy (Table 2, p = .05). The average postoperative speech rate was 97 words per minute (range 88 to 150 words per minute) and always allowed for normal social interaction.

Maximum phonation time is reported to measure adequacy of air support for speech and also reflects the adequacy of the laryngeal valve to act as a resistor to respiratory airflow for speech.[19] Maximum phonation time did not vary significantly after near-total laryngectomy in our studies. Since pulmonary status is not changed significantly due to surgery, such data suggested that the resistance of the near-total laryngectomy neoglottis is comparable to that of the preoperative glottis, resulting in no air loss during phonation. Such a feature results, in our opinion, from the preservation of a functional cricoarytenoid unit (superior laryngeal nerve, inferior laryngeal nerve, and cricoarytenoid joint and musculature) allowing for the creation of a self-contained tracheolaryngopharyngeal shunt that permits physiologic speech.

The average F0 after near-total laryngectomy — 162 Hz, in our study — is significantly higher than the preoperative F0 value (Table 2, p = .05). The postoperative SD of the F0 was significantly wider (Table 2, p = .05). Such data are in agreement with the report by Keith et al,[16] who noted higher than normal and more variable modal F0 values for sustained vowels in an analysis of 20 male speakers who had undergone near-total laryngectomy. Titze,[20] in an analysis of the control of F0 in normal speakers, stressed the relationship between the lung pressure and the vocal cords' length, the tension of the intrinsic laryngeal muscles, and the vocal cord–cover passive stress relationship. All these parameters, except lung pressure, were modified after near-total laryngectomy. The postoperative changes in frequency and acoustic perturbation measures were most likely the result of these modifications to the speech sound source. Another explanation could be the thicker vibrating portion of the neoglottis due to the plicature of the remaining true vocal cord, false vocal cord, and paraglottic space performed at the time of reconstruction. Such anatomic features also explain, in our opinion, the unstable vibrational pattern of the neoglottis, resulting in statistically increased shimmer and noise-to-harmonics postoperative values.

In conclusion, this preliminary prospective study provided quantitative data concerning speech and voice after near-total laryngectomy. Our data confirm the good quality of the voice and speech after near-total laryngectomy. In our series, the myomucosal pharyngeal flap described by Pearson et al[1,2] for enlarging the neoglottis was never used. Prospective studies are under way at our department 1) to further clarify the phonatory value of such a flap, 2) to evaluate the evolution of speech and voice parameters over time after near-total laryngectomy, and 3) to compare the speech and voice parameters achieved after near-total laryngectomy with the speech and voice parameters achieved after total laryngectomy with tracheoesophageal puncture and voice prosthesis insertion.

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KIMURA'S DISEASE: TWO CASE REPORTS AND A LITERATURE REVIEW

MARIA T. MESSINA-DOUCET, MD
NEW ORLEANS, LOUISIANA

WILLIAM B. ARMSTRONG, MD
IRVINE, CALIFORNIA

GLEN ALLISON, MD
IRVINE, CALIFORNIA

FRANCISCO PENA, MD
IRVINE, CALIFORNIA

JOYCE K. VALERA KIM, MD
IRVINE, CALIFORNIA

Kimura's disease (KD) is an allergic, inflammatory disorder of unknown cause. The typical presentation is in a young Oriental male with nontender subcutaneous swellings in the head and neck region, lymphadenopathy, peripheral eosinophilia, and elevated serum IgE. Many patients with KD also develop renal involvement. Treatment options range from conservative observation for asymptomatic patients to surgical excision, steroid therapy, and radiotherapy for symptomatic patients. Two cases of KD are presented. These emphasize not only the variability of disease presentation, but also the pitfalls of therapy leading to frequent recurrences. The literature is reviewed, summarizing patient presentation, differential diagnosis, current theories on causation, and therapy. A treatment protocol is also proposed.

KEY WORDS — glomerulonephritis, Kimura's disease, lymphadenopathy.

CASE REPORTS

Case 1. A 14-year-old Vietnamese boy presented initially to the Department of Nephrology at the University of California, Irvine, with a 3-year history of nephrotic syndrome and hypertension diagnosed in Vietnam. Two renal biopsies demonstrated segmental proliferative glomerulonephritis and mesangial proliferative glomerulonephritis. The patient was initially treated with prednisone and followed up bimonthly by measurements of 24-hour urine creatine and protein. The patient's urinary protein continued to rise. After a year of failed medical therapy, the patient was hospitalized in renal failure and peritoneal dialysis was initiated. Seven months later, the patient was referred to the Department of Otolaryngology for evaluation of a right preauricular mass, intermittently present since birth. The mass was firm to palpation, measuring 1 × 2 cm, with normal overlying skin. A 2 × 3-cm, firm submental mass was also identified, as well as a 2 × 3-cm right lateral neck mass. Left inguinal lymphadenopathy was also noted. A complete blood count demonstrated a white blood cell (WBC) count of 13.5 × $10^3/\mu L$ (normal, 4 to 10.5 × $10^3/\mu L$) with eosinophilia of 0.8 × $10^3/\mu L$ or 7.9% of lymphocytes (normal, 0 to 0.5 × $10^3/\mu L$). The patient underwent a right superficial parotidectomy and selective right neck dissection. On microscopic examination, both masses showed follicular and interfollicular hyperplasia, rich eosinophilic infiltration, and lysis of follicles. These findings were diagnostic of Kimura's disease (KD), and the patient was managed with conservative observation. Six months later, the patient returned with recurrent right cervical adenopathy. Physical examination revealed multiple nontender, nonfluctuant, firm nodes predominantly in the jugulodigastric chain. Some nodes were confluent, measuring 6 × 4 cm. Axillary and inguinal lymphadenopathy was also present. A complete blood count demonstrated a WBC count of 9.9 × $10^3/\mu L$ with eosinophilia of 1.3 × $10^3/\mu L$ or 13%, demonstrating persistent and rising eosinophilia. A repeat selective neck dissection was performed to excise the cervical mass and confirm the diagnosis of KD. Intraoperatively, the nodes were found to be confluent and easily removed from surrounding tissues. Histologically, the mass contained an eosinophilic infiltrate with both follicular and interfollicular hyperplasia. Many reactive follicles showed increased vascularization and eosinophilic abscesses. These findings are indicative of KD, thus confirming the original diagnosis.

Case 2. A 48-year-old healthy Vietnamese man presented with a 14-year history of a recurrent left preauricular mass (Fig 1A). The patient had undergone 2 previous attempts at local excision in Vietnam, with recurrence of the mass approximately 4

From the Departments of Otolaryngology–Head and Neck Surgery (Messina-Doucet, Armstrong, Allison) and Pathology (Pena, Kim), University of California, Irvine, California.

CORRESPONDENCE — Maria T. Messina-Doucet, MD, 2020 Gravier St, Suite A, New Orleans, LA 70112-2234.

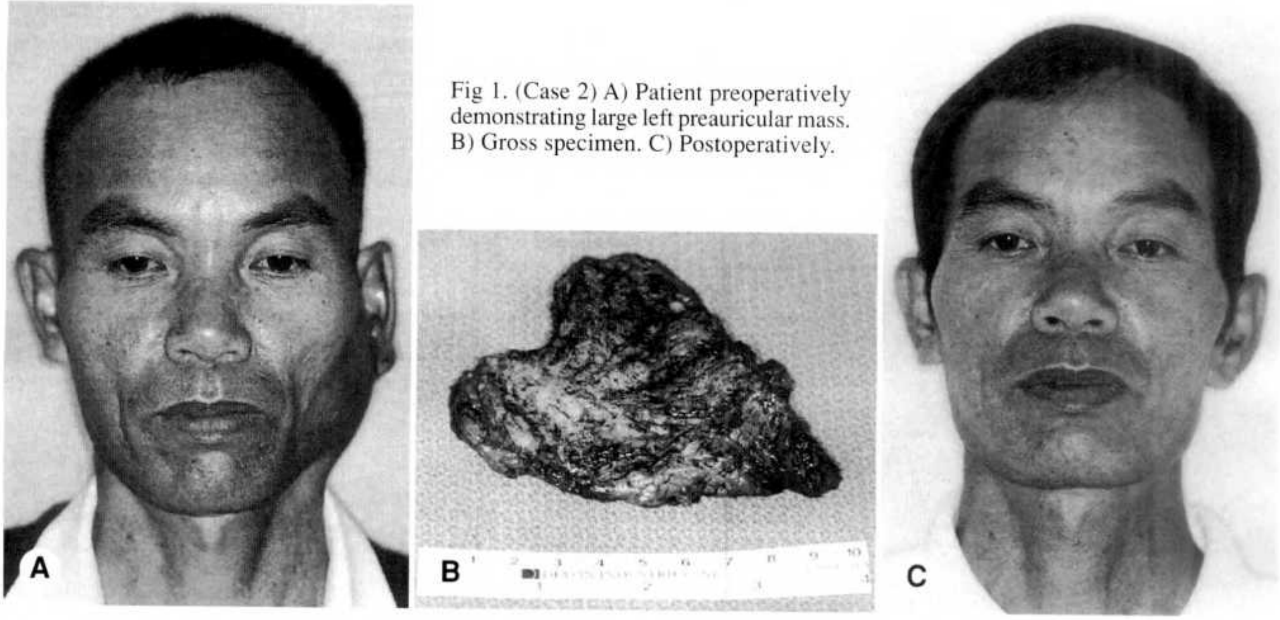

Fig 1. (Case 2) A) Patient preoperatively demonstrating large left preauricular mass. B) Gross specimen. C) Postoperatively.

years after each excision. Physical examination revealed a 5 × 7-cm indurated mass overlying the left masseter muscle, angle of the mandible, and postauricular area with mild erythema of the overlying skin. The facial nerve was intact. Multiple lymph nodes measuring less than 1 cm were palpable anterior to the left sternocleidomastoid muscle. Two fine needle aspirations were performed. The first aspirate showed benign glandular epithelium and mature brown fat. Repeat needle aspiration 1 month later showed reactive lymphoid tissue. Laboratory studies revealed a WBC count of $8.3 \times 10^3/\mu L$ and eosinophilia of $3 \times 10^3/\mu L$ (22%). A left superficial parotidectomy and selective neck dissection was performed. The mass was extensively vascular, rubbery, fibrotic, and adherent to the overlying dermis (Fig 1B). The deep parotid gland and facial nerve were not involved. Histologically, the excised tissue showed reactive lymphoid hyperplasia and fibrosis, and acute and chronic inflammation with marked eosinophilia. The diagnosis of KD was made.

DISCUSSION

Investigations of benign lymph node enlargement with eosinophilic infiltrate were first described in China in 1937 by H. T. Kimm and C. Szeto.[1,2] In 1948, Kimura et al[3] published a paper describing an "unusual granulation and hyperplastic changes of lymphatic tissue," and from that point on, the disease became known as Kimura's disease. Today, KD is felt to be an allergic inflammatory disorder of unknown cause. The typical presentation is in a young Oriental male with nontender subcutaneous swellings in the head and neck region, predominately in the preauricular and submandibular areas. Rare cases have been reported to include the scalp and orbital area.[4] Lymphadenopathy, both local and distant, peripheral eosinophilia, and an elevated serum IgE are also present. The disease usually follows a benign, indolent course. The only frequently reported systemic manifestation has been renal involvement, leading to the nephrotic syndrome in up to 60% of patients.[1,2,5,6]

A case report by Kuo et al[2] demonstrated a patient with known KD to have involvement of the skeletal muscles and the prostate gland. This suggests that perhaps microscopic involvement is more systemic than previously appreciated.

Several entities may mimic KD, and a differential diagnosis should include angiolymphoid hyperplasia with eosinophilia (ALHE), Kaposi's sarcoma, tuberculosis, nodal metastasis (breast and rectal cancer), eosinophilic granuloma, hamartoma, lymphoma, lymphocytoma, epithelioid hemangioma, angiofollicular hyperplasia, low-grade angiosarcoma, parotid tumor, and Mikulicz's disease.[2,7-10]

One of the most controversial differential diagnoses in the literature has been between ALHE and KD: whether they are distinct entities or one and the same. Recent literature describes these as 2 distinct entities.[4,7,8,11] The clinical differences are summarized in the Table.[7,8,11,12]

Although significant effort has been focused on differentiating these 2 entities, the difference is of little clinical significance, as conservative surgery is the treatment of choice for both conditions.[7]

Computed tomography (CT) and magnetic resonance imaging (MRI) have been useful in the investigation of KD. Som and Biller[9] demonstrated the usefulness of CT and MRI in their case report of a pa-

SUMMARY OF CLINICAL DIFFERENCES BETWEEN KIMURA'S DISEASE AND ANGIOLYMPHOID HYPERPLASIA WITH EOSINOPHILIA

	Kimura's Disease	*ALHE*
Nationality	Oriental	Western
Age of incidence	2nd to 3rd decade	3rd to 5th decade
Gender preference	Male predominance	Equal
Lesion	Large nodule or tumor (usually >2 cm and deep)	Papule or nodule (usually <2 cm and superficial)
Overlying skin	Normal	Erythematous to brown
Lymphadenopathy	Common	Uncommon
Duration of history	2 mo to 10 y	3 wk to 12 y
Serum IgE	Usually elevated	Usually normal
Eosinophilia	Usually >10%	Usually <10%

Information is from references 7, 8, 11, and 12.

tient with known KD involving the parotid gland and cervical lymph nodes. A CT scan showed a contrast intensity in the parotid gland and ipsilateral lymph nodes. The enhancement of the involved areas was similar in intensity to the internal carotid artery, suggesting the vascular nature of the disease. On MRI, both the parotid mass and involved lymph nodes demonstrated high T1- and T2-weighted signal intensities.[9] Although imaging studies are not diagnostic, they may help delineate the extent of disease.

The diagnosis of KD is often difficult, requiring biopsy or excision of the involved lymph node for a pathologic diagnosis. Histologically, the tissues show reactive prominent germinal centers composed of 3 elements: cellular, fibrocollagenous, and vascular.[6,11,13] The cellular component consists of dense eosinophilic infiltrate of the interfollicular zones, lysis of follicles, and microabscesses (Fig 2A). Granuloma formations contain infiltrates of eosinophils, lymphocytes, plasma cells, and histiocytes. Dense tissue fibrosis and vascular proliferation are present (Fig 2B). Vessels remain thin-walled, with cuboidal endothelial cells.[2,6,11] Polykaryocytes of the Warthin-Finkeldey type can be demonstrated, but are not pathognomonic of the disease (Fig 2C).[2,6] Immunofluorescence studies show germinal centers containing heavy IgE deposits and variable amounts of IgG, IgM, and fibrinogen.[2,13]

As previously noted, KD is often confused with ALHE, which also causes a rich eosinophilic infiltration. Distinct histologic differences between KD and ALHE can be noted. "Histiocytoid" blood vessels, seen in ALHE, have never been found in KD. These are described as containing hypertrophied endothelial cells with eosinophilic cytoplasm. Some are characteristically vacuolated. Vacuolated endothelial cells are never seen in KD.[2,11] The other major histopathologic difference is that the germinal centers of KD can become so heavily infiltrated with eosinophils that progressive destruction ensues, causing lysis of the follicles. Although lysis of follicles can be found in almost every case of KD, it has never been seen in ALHE.[2,11] The third difference is that immunohistochemical stains have shown IgE in the germinal centers of KD but not ALHE.[13]

The cause of KD remains unknown, but several causes have been speculated. These include interference with immune regulation, atopic reaction to a continuous antigenic stimulus (specifically, *Candida albicans*), parasitic infection, and neoplasm.[14-19] The general consensus is that KD is a chronic inflammatory condition.

Terada et al[14] have attempted to gain a better understanding of KD by studying eosinophil activation and infiltration. They propose that *C albicans* may be the agent responsible for the peripheral blood eosinophilia and eosinophilic infiltrate into lymph nodes. Interleukin-5 (IL-5) is known to be a potent, selective stimulator of eosinophil activation, differentiation, proliferation, and chemotaxis. In their study, when a site of granuloma was stimulated with *Candida* antigen, IL-5 was produced and released. Interleukin-5 gene expression was also amplified.[14,17] The following mechanism of eosinophilic infiltration was recommended. Locally produced IL-5 causes an eosinophilic infiltration into the granuloma from the blood vessels. Once the eosinophils migrate to the inflammatory site, they are activated by IL-5 to increase cell density. These eosinophils are then saved from programmed cell death and survive for longer periods than normal eosinophils. This produces the accumulation of eosinophils at the local site of inflammation and in the peripheral blood. This corresponds with the fact that the peripheral blood of patients with KD shows a large proportion of hypodense eosinophils with prolonged viability.[14]

The work of Terada et al[14] suggests that IL-5 production induced by *Candida* contributes to the eosinophilia of KD. Although this has been demonstrated in laboratory studies, human investigations have not been done. *Candida albicans* has not been isolated in lesions of KD, and investigations have not addressed whether other antigens could induce the same response.

The role of eosinophils in allergic disease is well known. Eosinophils contain cytotoxic proteins in their granules, such as eosinophilic cationic protein (ECP) and major basic protein (MBP). Both ECP and MBP have been related to bronchial asthma, acting upon mast cells and causing histamine release. Morita and Kitano[16] found that in the active stage of

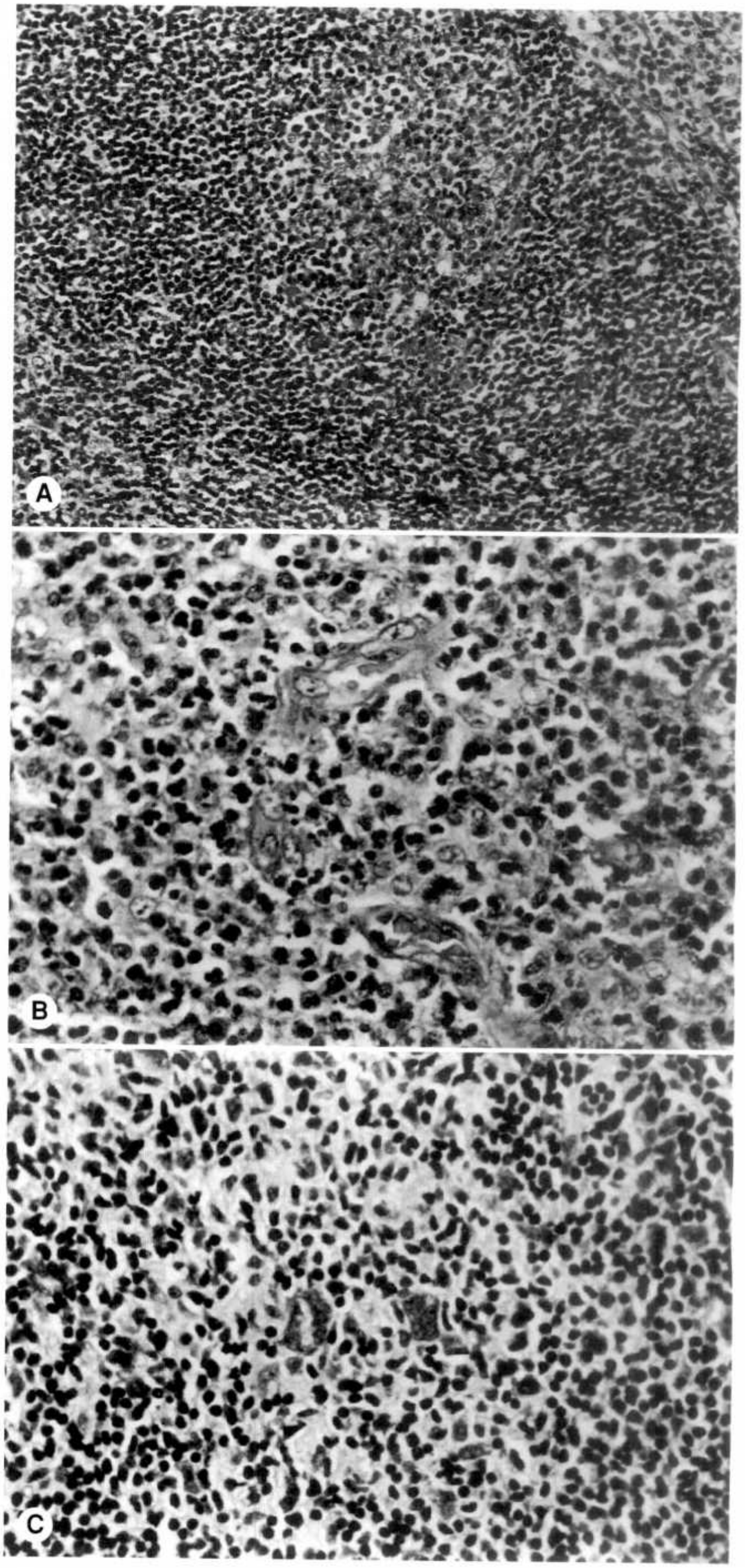

Fig 2. Histology of Kimura's disease. A) Involuted germinal center with eosinophilic microabscess in center and parafollicular infiltrate of eosinophils (H & E, original × 250). B) Proliferation of venules in interfollicular area with surrounding eosinophils (H & E, original ×450). C) Warthin-Finkeldey polykaryocytes in germinal center (H & E, original ×450).

KD, ECP and MBP were higher than in controls, and that they decreased during disease remission. Therefore, ECP and MBP may also play a role in the development of KD.[16]

Immunohistochemical studies have been performed to determine changes in T cell activity in KD. Tabata et al[15] performed these studies on skin and lymph nodes of a patient with KD and showed active proliferation of HLA-DR+ CD4+ cells. Two-color flow cytometry confirmed the active CD4 cells in the lymph and peripheral blood. Activation of helper T cells is known to augment B cell function. Thus, proliferation of CD4 cells may precipitate the high IgE and lymphoid follicle production that is seen in KD. Therefore, the proliferation of CD4 cells may suggest a neoplastic or T cell proliferative disorder as a cause of KD.[15]

The mechanism of renal disease is also being investigated. It is unlikely to be secondary to substances released from the soft tissue lesions, as some cases of KD present with proteinuria prior to the appearance of subcutaneous lesions.[5] As is suspected for the subcutaneous lesions, a systemic allergic reaction could also be involved in the renal disorder. The constellation of symptoms — elevated serum IgE, lymph proliferation, and eosinophilia — could all account for glomerulopathy. Histologically, membranous glomerulonephropathy, minimal change disease, diffuse proliferative glomerulonephritis, and mesangial proliferative glomerulonephritis have been demonstrated on renal biopsy in patients with proteinuria and KD.[19]

Perhaps a persistent antigenemia causing IgE deposits along the glomerular capillary wall causes epithelial damage to renal glomeruli, resulting in proteinuria.[6,20] Immunoglobulin E deposits have been reported by immunofluorescence along the capillary wall and paramesangial areas.[19,20] Case reports also demonstrated marked podocyte detachment and epithelial bleb formation.[5,6,14,19,20] Such paramesangial destructive lesions may correlate with the activity of glomerulonephritis and the severity of proteinuria. When the allergic state is active, with elevated IgE and eosinophilia, active glomerulonephritis is present. Regression of proteinuria is seen with control of the allergic state.[20]

Although there are many speculations about the cause of KD, the true cause still remains unknown. An etiologic agent such as a bacteria, parasite, or fungus has never been isolated in lesions of KD.[19] The most prevalent theory in the literature is that of *Candida* acting as the source of persistent antigenemia, even though neither hyphae or spores have ever been isolated from involved tissues. Takenaka et al[19] suggest that the disease may be caused by the absorption of soluble substances, rather than the growth of the fungus, that act as the source of antigenemia. Other investigators have identified specific IgE antibodies to *C albicans* in patients with KD, as well as a positive immediate skin reaction to *Candida* extract.[6,12,19] These studies further the suspicion that KD results from an atopic reaction to *Candida.*

The optimal treatment of KD is not well defined in the literature. Surgical excision is the most common diagnostic measure and is often also therapeutic. Management options for KD include surgical excision, systemic steroid therapy, radiotherapy, and observation. The treatment outcome is variable, and recurrence is common, occurring in up to 25% of cases treated with surgical excision alone.[1]

After diagnosis by surgical excision, recurrent masses may be managed with steroid therapy. Patients are started on a high dose of prednisone, and the dose is tapered to the minimal dose that will suppress recurrence. Long-term steroid treatment can have many side effects that limit its use.[5,6,20]

Concern for secondary malignancy has dampened enthusiasm for the use of radiotherapy for this usually benign disease. However, 80% control (8 months to 13 years) with 25 to 30 Gy of local irradiation has been reported.[9,12,20] Secondary malignancy in fields irradiated for KD has not been reported, although studies are limited.[12,20] Indications for radiotherapy include patients who have failed medical management, with masses refractory to steroid treatment or tumor relapse after steroid withdrawal. The advantage of radiotherapy for KD is that it offsets the need for long-term corticosteroids with the associated risks and side effects. Moderate doses of radiation provide local control, alleviating the need for steroid maintenance.

A summary of recent reports on the treatment of KD suggests the following treatment protocol. At initial presentation, surgical excision is the treatment of choice for definitive diagnosis and initial management.[2,8,10,20] Once the diagnosis is made, the patient should be screened for renal involvement by checking urinary protein levels. The patient should then be followed conservatively for recurrent disease. Localized initial recurrence can often be managed by surgical excision. If recurrence becomes frequent or the patient develops symptomatic nephrotic syndrome, medical management with steroids should be initiated. As in case 1, patients with nephrotic syndrome should have renal function followed closely by a nephrologist, with laboratory studies including bimonthly 24-hour urine protein excretion. The dose of steroids used to manage the proteinuria may fluctuate, but treatment should be initiated with high-dose prednisone.[5,6,20] Steroid dosages are tapered to effect. In patients failing medical management of the nephrotic syndrome or with tumors failing to regress, radiotherapy should be considered.[9,20] In patients with severe renal disease and podocyte detachment, aggressive treatment with corticosteroid, cyclophosphamide, and antiplatelet drugs has been recommended to help preserve renal function.[20] Sometimes, even with this aggressive treatment plan, complete remission is not possible.

CONCLUSION

Kimura's disease is a rare entity causing subcutaneous swellings of the head and neck. The purpose of reviewing this disorder is to draw attention to this disease, as its presentation could easily cause it to be confused with malignancies. Conservative therapy is warranted, as KD usually follows an indolent course. As the diagnosis is made pathologically, surgical excision is the treatment for diagnosis and initial management. Renal involvement should be excluded by checking urinary protein levels. A protocol for treating recurrent and systemic disease has been proposed. Further investigations into the cause of KD are warranted in order that therapy may be directed against the etiologic agents rather than the symptoms of the disease.

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RETROPHARYNGEAL ABSCESS AND EPSTEIN-BARR VIRUS INFECTION IN CHILDREN

THOMAS G. TAKOUDES, MD JOSEPH HADDAD JR, MD

NEW YORK, NEW YORK

A 6-year retrospective chart review was conducted of all pediatric patients with the diagnosis of retropharyngeal abscess (RPA) at the Columbia-Presbyterian Medical Center. The charts were examined for signs, symptoms, and serologic findings consistent with recent acute Epstein-Barr virus (EBV) infection. Of the 7 patients we studied, 4 had elevated immunoglobulin G antibodies that were consistent with recent acute EBV infection, and 3 patients were not tested. The 4 patients with positive titers also presented with signs typical for acute EBV infection, including fever (3/4), lymphadenopathy (4/4), and pharyngitis (4/4). Of note, the 2 patients who were older than expected for RPA (ages 18 and 11) had the most severe infections. We submit that there may be a role for EBV infection in the pathogenesis of RPA formation in children. Increasing age may correlate with a more severe infection in this setting.

KEY WORDS — Epstein-Barr virus infection, neck infections, retropharyngeal abscess.

INTRODUCTION

Retropharyngeal abscess (RPA) is a rare complication of head and neck infections in the pediatric population; RPAs typically occur in children after an acute upper respiratory tract infection.[1] Infections of the nose, nasopharynx, paranasal sinuses, adenoids, and eustachian tubes drain to retropharyngeal lymph nodes,[2] which may cause retropharyngeal lymphadenitis and RPA formation. Because these nodes regress by 4 or 5 years of age, most RPAs occur in children younger than 5 years old.[3] Although acute primary Epstein-Barr virus (EBV) infection is relatively common and is known to cause lymphadenitis in children,[4] there is no evidence in the literature of a correlation between RPA and EBV. Accordingly, a 6-year retrospective chart review of all cases of RPA at this medical center was conducted.

METHODS

A retrospective chart review was conducted for the 6-year period from January 1989 to January 1995. The case records of the 8 pediatric patients who were treated for RPA at Columbia-Presbyterian Medical Center were retrieved and evaluated. The diagnosis of RPA was based on clinical, radiologic, and operative findings.

The records were analyzed for clinical presentation, physical findings, and laboratory data, as well as for a history of previous EBV exposure. In particular, signs, symptoms, and laboratory findings consistent with acute primary EBV infection were assessed. These included fever, adenopathy, pharyngitis, EBV serologies, and other laboratory data.

A patient was considered to have acute primary EBV infection if a heterophil antibody test was positive or if the patient had an immunoglobulin G (IgG) antibody to EBV capsid antigens of 1:320 or more.[5] A negative heterophil antibody test was considered nondiagnostic, because this test carries a 10% to 15% false-negative rate.[4,6,7]

RESULTS

Eight patients were treated at Columbia-Presbyterian Medical Center for RPA between 1989 and 1995. One patient was excluded from this study because of a complicated medical history including a branchial cleft cyst and multiple neck operations. The mean age was 5.8 years, the mode age was 3 years, and the range was 13 months to 18 years. There was a male preponderance of 5 cases to 2 cases. Imaging studies consisted of lateral neck radiography and/or computed tomography of the neck in order to assess prevertebral soft tissue thickness, soft tissue collections, and adenopathy. Figures 1 and 2 from patient 1 show typical radiologic findings of RPAs.

Treatment consisted of incision and drainage of the abscess combined with intravenous antibiotics in 5 patients and intravenous antibiotics alone in 2 patients. None of the patients had known previous episodes of EBV infection prior to their hospitalization for treatment of their RPAs. The results of serologic and microbiologic testing for the 7 patients ana-

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From the Division of Pediatric Otolaryngology–Head and Neck Surgery, Babies and Children's Hospital of New York, Columbia-Presbyterian Medical Center, New York, New York.

CORRESPONDENCE — Joseph Haddad Jr, MD, Division of Pediatric Otolaryngology–Head and Neck Surgery, Babies and Children's Hospital of New York, Columbia-Presbyterian Medical Center, Babies Hospital North 5-501, New York, NY 10032.

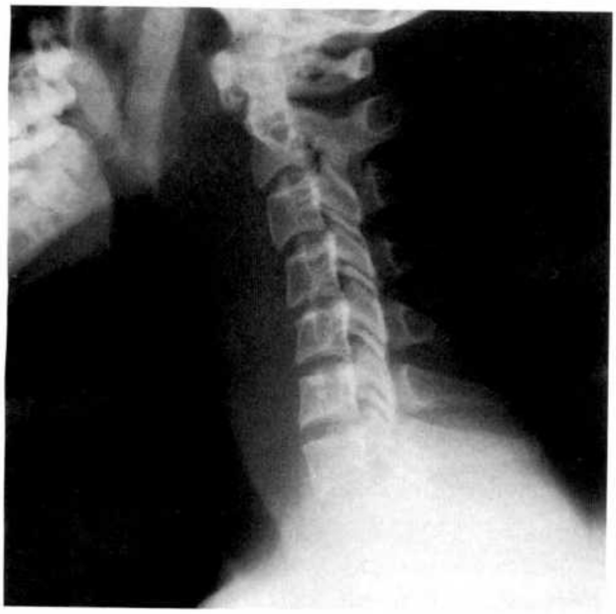

Fig 1. (Patient 1) Lateral neck radiograph demonstrating thickened prevertebral soft tissue consistent with retropharyngeal abscess.

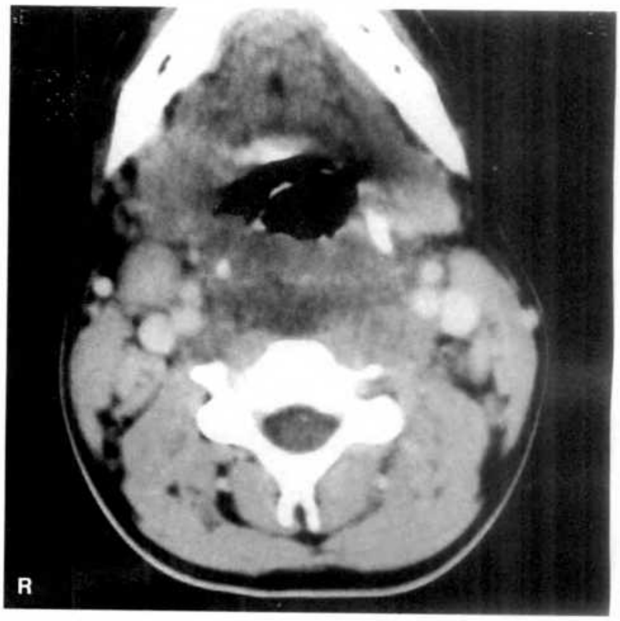

Fig 2. (Patient 1) Computed tomography scan of neck. This image shows large retropharyngeal abscess, as well as significant associated lymphadenopathy.

lyzed are shown in the Table. Of the 7 patients in this study, 4 showed strong evidence of recent acute primary EBV infection based on history and elevated IgG titers. The remaining 3 patients had inconclusive data to determine EBV status.

Patients With Acute Primary EBV Infection. Four patients had elevated IgG titers suggestive of recent EBV infection. Patient 1, an 18-year-old, had a titer change from negative to 1:640 during a 3-week period that encompassed his evaluation. In addition, he had a positive heterophil antibody test. He was taken to the operating room, where 40 mL of purulent discharge was drained. Patient 2 (age 3) had a 1:2,560 titer and responded to intravenous antibiotic therapy. Patient 3 and patient 4 each had a titer of 1:320. Patient 3 was an 11-year-old who had 10 mL of purulent discharge drained in the operating room. Patient 4 was a 13-month-old with 1 mL of pus drained at surgery.

These 4 patients also showed signs typical of EBV infection, including fever (3/4), lymphadenopathy (4/4), and pharyngitis (4/4).

Patients With Inconclusive Data. Three patients in the study did not have sufficient tests to accurately assess the possible diagnosis of acute primary EBV infection. None of these patients had an EBV IgG titer level determined. Patient 7 had a negative heterophil antibody test, which was considered nondiagnostic. Two of these patients had 1 mL of purulent

PERTINENT LABORATORY VALUES AND PHYSICAL FINDINGS IN SEVEN PATIENTS TREATED FOR RETROPHARYNGEAL ABSCESS

Pt No.	*Age (y)*	*IgG Titer**	*Heterophil*	*Fever*	*Lymphadenopathy*	*Pharyngitis*	*Treatment*	*Abscess Culture*
1	18	1:640	Positive	−	+	+	I&D, 40 mL	*Fusobacterium*, coagulase-negative *Staphylococcus*
2	3	1:2,560	NT	+	+	+	Antibiotics	NT
3	11	1:320	NT	+	+	+	I&D, 10 mL	Group A *Streptococcus*
4	1	1:320	Negative	+	+	+	I&D, 1 mL	*Streptococcus*
5	3	NT	NT	+	+	+	Antibiotics	NT
6	2	NT	NT	+	+	+	I&D, 1 mL	*Bacteroides melaninogenicus*
7	3	NT	Negative	+	+	+	I&D, 1 mL	*Streptococcus viridans*

I&D — incision and drainage, NT — not tested.

*To Epstein-Barr virus capsid antigen.

drainage removed at surgery, and 1 was treated with antibiotics alone. Signs and symptoms typical for acute EBV infection were found in all 3 patients in this group: fever, lymphadenopathy, and pharyngitis.

DISCUSSION

This study examined 7 patients with RPAs for evidence of acute EBV infection during a 6-year period at the Columbia-Presbyterian Medical Center. It is relevant that 4 of the 7 patients treated for RPA demonstrated strong evidence of acute primary EBV infection. This evidence is based on physical signs as well as serologic tests. It should be noted, however, that while fever, lymphadenopathy, and pharyngitis often occur in acute EBV infection, they may occur with RPA formation alone. The data on the 3 remaining patients are inconclusive because of incomplete laboratory tests. Although peritonsillar abscess is a known rare complication of EBV infection,[8,9] this is the first study to demonstrate a correlation between RPA formation in the pediatric population and acute EBV infection.

The retropharyngeal space needs to be differentiated from the danger space and the prevertebral space. Although some controversy exists, the retropharyngeal space is thought to lie between the visceral division of the middle layer of the deep cervical fascia anteriorly and the alar division of the deep layer of the deep cervical fascia posteriorly. It extends from the base of the skull to the first or second thoracic vertebra. The danger space is thought to be posterior to this, specifically between the alar and prevertebral divisions of the deep layer of the deep cervical fascia. Posterior to this is the prevertebral space, which lies anterior to the vertebral bodies. This space extends throughout the length of the vertebral column.[3]

Some controversy does exist about the nomenclature, as many contemporary head and neck anatomists consider the retropharyngeal space to be synonymous with the danger and prevertebral spaces. This is based on a report that the latter 2 spaces do not exist as separate entities and that the alar layer of prevertebral fascia does not exist.[10]

Retropharyngeal abscesses are typically seen in 1 of 3 situations[1]: 1) as acute suppuration in a retropharyngeal lymph node after an upper respiratory tract infection, occurring in children more often than in adults; 2) after perforation of a foreign body through the posterior pharyngeal mucosa; or 3) secondary to direct extension of tuberculosis of the cervical spine through the anterior longitudinal ligament. The patient usually complains of any combination of fever (88%), neck swelling (62%), anorexia (51%), and throat pain (49%). Typical physical findings include cervical adenopathy (69%), retropharyngeal bulge (43%), tonsillitis and peritonsillitis (28%), and pharyngitis (12%).[10] Although children classically develop RPAs after recent upper respiratory tract infections,[1,3,11] the nature of these respiratory tract infections is poorly characterized.

Common infecting organisms include α-hemolytic streptococci, *Neisseria* species, and *Staphylococcus aureus*.[11,12] Culture results from the present study are shown in the Table. All abscesses that were tested grew positive cultures. There does not appear to be an association between the organism recovered and the presence or absence of acute EBV infection.

Primary EBV infection is common among young children and is the leading cause of infectious mononucleosis.[4] In children, acute EBV infection is generally asymptomatic or accompanied by nonspecific manifestations such as fever, lymphadenopathy, and pharyngitis. In addition, typical but less common signs such as splenomegaly, hepatomegaly, and abdominal pain may be present.[13]

Diagnosis of acute primary EBV infection can generally be confirmed with a positive heterophil antibody test.[5] A negative heterophil test requires further evaluation, since the test carries a 10% to 15% false-negative rate[4,6] in adults and an even higher rate in children (up to 94% in children less than 2 years old in one study[7]). Additional serologic tests that are useful for the diagnosis of acute EBV include tests for IgM and IgG antibodies that form in response to EBV capsid antigens. The IgM antibodies are present during the acute phase of EBV infection,[7] and an IgG antibody level greater than 1:320 is consistent with a presumptive diagnosis of acute EBV infection.[5] Patients who have recovered from previous EBV infection typically have lifelong IgG titers of 1:10 to 1:80.[5] Antibodies to early antigens may also be useful in the diagnosis of acute infection. These include anti-D antibody, which is found in 70% of patients with acute infectious mononucleosis and persists for 3 to 6 months.[4] Antibodies to Epstein-Barr nuclear antigen (EBNA) appear late in the course of EBV infection and persist throughout life. The appearance of antibodies to EBNA in a patient who was previously viral capsid antigen–positive and EBNA-negative is strong evidence of recent EBV infection.[4]

Serum analysis was limited in this study, because it was a retrospective chart review. However, future testing of patients with RPAs who are suspicious for acute EBV infection should include assays that are useful in determining if the infection is acute. These include tests for heterophil antibodies, IgG and IgM antibodies, and antibodies to early antigens and EBNA.

The relationship between RPA and EBV may be secondary to the mechanisms of EBV infection. The virus is acquired by contact with infected secretions and preferentially infects pharyngeal epithelial cells and B lymphocytes.[6] The acute phase of the infection typically causes lymphadenopathy, pharyngitis, fever, and malaise.[4] We postulate that retropharyngeal lymph nodes that drain portions of the upper respiratory tract may become inflamed in severe cases. If acute inflammatory reactions of lymph nodes are not abated, acute suppurative lymphadenitis and abscess formation may occur.[14] While this appears to be a rare complication of acute EBV infection, the potential for morbidity and mortality is significant, as it may lead to upper airway obstruction or aspiration of a ruptured abscess.[9]

The age of the patient may play a role in the clinical presentation. It was noted that 2 of the 7 patients were 11 and 18 years old. These 2 patients are interesting for 2 reasons. First, they are older than expected for RPA formation. A series of 65 patients with this type of abscess noted that half developed the abscess prior to 3 years of age, and 71% before 6 years of age.[10] This finding is probably secondary to the fact that retropharyngeal nodes regress before the age of 5.[3] Second, these 2 patients had the greatest amount of pus drained from their abscesses. Specifically, 40 mL of pus was drained from the 18-year-old, and 10 mL was drained from the 11-year-old. This is a greater quantity than the 1 mL drained from the 3 other patients who were treated with incision and drainage. These findings are consistent with the typically more severe clinical presentation of acute EBV infection in adolescents compared to younger children, including tonsillopharyngitis and adenopathy.[13]

Although this study yielded some interesting information, it is limited in its design. A retrospective chart review does not allow for strict control of patient evaluation and treatment. In addition, the number of patients in this study is small. With only 8 subjects, the ability to distinguish a true correlation from a chance occurrence is diminished. Data analysis, however, is suggestive of a correlation between EBV and RPA, and further investigations may be helpful in understanding this relationship.

We conclude that there may be a role for EBV infection in the pathogenesis of RPA formation in children. Age may be related to the severity of the clinical presentation, including the size of the abscess. The possibility of acute EBV infection in a patient with RPA has diagnostic and therapeutic implications. If the recovery from the symptoms of an abscess is slow, then recent EBV infection should be considered, especially if the child is more than 6 years old. The previously discussed serologic tests should be performed to determine the patient's EBV status and thereby to aid in decisions about therapeutic interventions.

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IMAGING CASE STUDY OF THE MONTH

COCHLEAR OTOSCLEROSIS: THE CURRENT UNDERSTANDING

OLIVER YOUSSEF, MD
SUJANA CHANDRASEKHAR, MD
ARIE ROSEN, MD
HUEY-JEN LEE, MD

NEWARK, NEW JERSEY

INTRODUCTION

It is widely accepted that clinical otosclerosis can cause some degree of sensorineural hearing loss (SNHL) in addition to conductive hearing loss. However, controversy exists as to whether or not pure SNHL can be caused by isolated cochlear otosclerosis (CO) without stapedial involvement. Histologic studies of temporal bones have shown that in some patients with conductive hearing loss and clinical otosclerosis, a second focus of otosclerosis can be found in the cochlea.[1-3] Histologic evidence of pure CO is very rare: Schuknecht and Barber[2] found isolated involvement of the cochlea without stapes fixation in only 5 of 164 bones with otosclerosis. In only 2 of the bones were they able to link the SNHL to the pathologic findings. Balle and Linthicum[3] documented histologic evidence of isolated CO without stapedial involvement in 7 temporal bones obtained from 5 individuals with pure SNHL. Their report offers probably the most compelling histologic evidence in support of the existence of isolated CO in the literature. Histologic studies of temporal bones do not necessarily reflect the real incidence of CO, because patients with SNHL are usually assumed to have presbycusis and are therefore not asked to will their temporal bones.

Early attempts to image otosclerosis utilized polytomography. Polytomography had a high sensitivity in demonstrating surgically proven stapedial otosclerosis (90% to 100%); however, the specificity of a positive test was only 71%. The ability of polytomography to predict cochlear involvement was even poorer, with 40% of the cochlear lesions not identified (false negatives).[4] More recent studies applied high-resolution computed tomography (CT) to the diagnosis of CO.[5-9] The cochlear capsule is normally a very dense and compact bone with clearly defined margins. Its boundaries can be easily distinguished from the surrounding bone because of its maximal CT density characteristics. Mafee et al[6] reported CT changes in the otic capsule that they attributed to cochlear involvement in 18 of 19 patients (sensitivity of 95%). All 19 patients had clinical stapedial otosclerosis with a mixed hearing loss.

Magnetic resonance imaging (MRI) is sensitive in demonstrating the membranous labyrinth and the neural components of the internal auditory canal. It is currently the study of choice for retrocochlear workup of SNHL. In order to identify CO on MRI, one has to be aware of the characteristic findings of CO on MRI. There have been very few reports on MRI characteristics of CO. We report a case of a 48-year-old woman with bilateral CO whose diagnosis was initially suggested by MRI and subsequently confirmed by high-resolution CT.

CASE REPORT

A 48-year-old woman was evaluated for a progres-

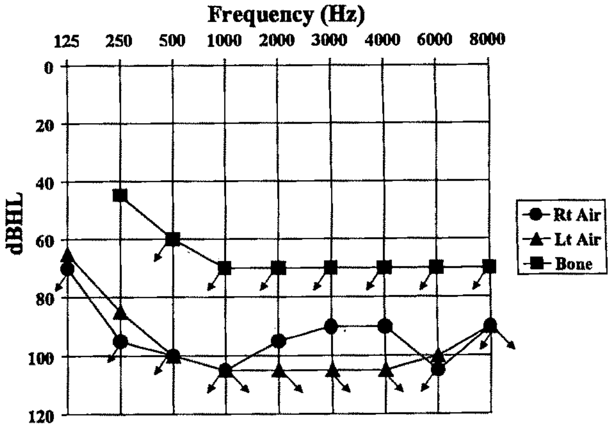

Fig 1. Patient's audiogram showing bilateral profound asymmetrical sensorineural hearing loss.

From the Section of Otolaryngology–Head and Neck Surgery (Youssef, Rosen, Chandrasekhar), and the Department of Radiology (Lee), New Jersey Medical School, Newark, New Jersey.

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CORRESPONDENCE — Arie Rosen, MD, Section of Otolaryngology–Head and Neck Surgery, New Jersey Medical School, 185 S Orange Ave, H-592, Newark, NJ 07103.

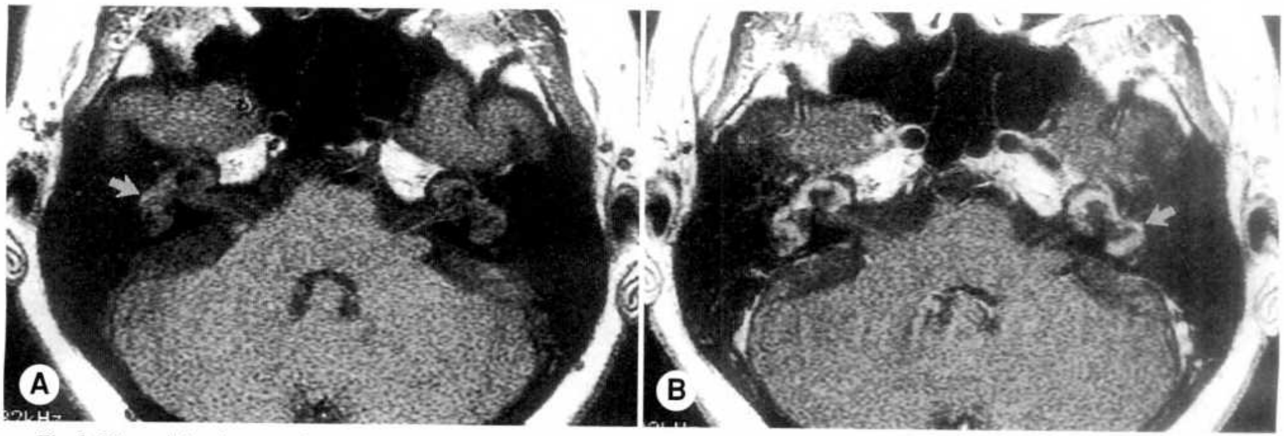

Fig 2. T1-weighted magnetic resonance images. A) Without gadolinium enhancement, showing bilateral faint pericochlear and perilabyrinthine hyperintensity (arrow) similar to that of adjacent soft tissue. B) With gadolinium, showing bilateral pericochlear and perilabyrinthine enhancement (arrow).

sive bilateral SNHL and occasional vertigo and tinnitus in the left ear. Her hearing had deteriorated since she was 18, with each of her 3 pregnancies. She had been without hearing in her left ear for the past 4 years and used a hearing aid for her right ear. On audiologic evaluation she had a profound SNHL in the left ear and a severe to profound SNHL in the right ear (Fig 1). Tympanometry revealed normal middle ear pressure and compliance bilaterally. She had no history of osteogenesis imperfecta or syphilis and no family history of otosclerosis. The patient's speech and ear examination findings were normal. Pertinent laboratory findings were also normal (fluorescent treponemal antibody absorption and erythrocyte sedimentation rate).

Temporal bone MRI with gadolinium was performed for retrocochlear workup of the patient's SNHL. It revealed pericochlear and perilabyrinthine gadolinium enhancement on T1-weighted images (Fig 2). On these images, the lesion appeared similar in density to soft tissue. These MRI findings suggested the presence of CO, and high-resolution CT was subsequently obtained that confirmed the diagnosis (Fig 3). It displayed a remarkably extensive lucency of the entire otic capsule bilaterally. Left middle ear exploration subsequently revealed a mobile stapes.

DISCUSSION

In pure CO, parts of the endosteal layer of the cochlea are replaced by otosclerotic changes without stapes footplate fixation. Patients present with pure SNHL. The condition is both rare and difficult to diagnose. It is particularly difficult to clinically differentiate CO from early-stage presbycusis. The diagnosis should be suspected when any or all of the following clinical conditions are present: unexplained SNHL at a young age, progressive SNHL, history of surgery for otosclerosis, or a family history of otosclerosis.

Otosclerosis has several distinct stages: in the early stage, it is characterized histologically by the enlargement of perivascular spaces, osteoclastic bone resorption, and new immature bone formation within the endochondral bone layer of the otic capsule. In the later stage, dense sclerotic bone may be depos-

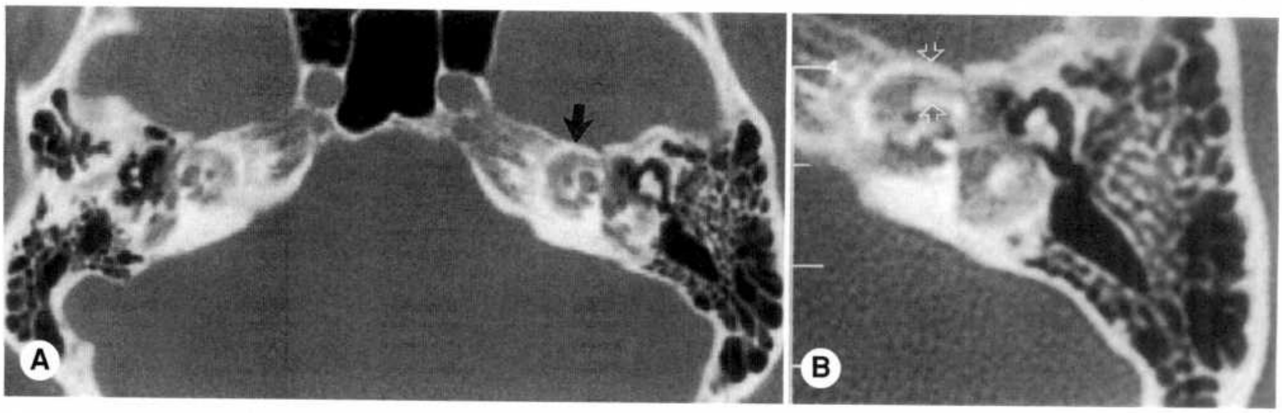

Fig 3. Computed tomograms. A) Depicting extensive lucency of cochlea and vestibule bilaterally (arrow). B) Of right temporal bone, showing cochlear and vestibular demineralization creating so-called double-ring effect (arrows).

ited. The pathologic findings of otosclerosis may therefore vary widely between dense, "sclerotic" bone and lytic, "spongiotic" bone.[6] Spongiotic lesions are hypercellular, vascular, and less dense when compared to the normal or sclerotic otic capsule. The larger the otosclerotic foci become, the more vascular and more active they become. Large foci of otosclerosis (>4 mm) show extensive bone resorption and replacement by fibrous and vascular tissue that eventually can undergo cavitation.[2]

High-resolution CT is the imaging modality of choice for the diagnosis of otosclerosis. It can detect abnormalities of the oval window area in 80% to 90% of patients with surgically proven otosclerosis.[2,5,6] The CT findings of otosclerosis are characterized by lucent (demineralized) foci, which may be single, multiple, or confluent. Extensive confluent spongiotic cochlear foci appear as a pericochlear lucency or a double-ring effect on CT.[7,8] This characteristic finding was noted on our patient's CT (Fig 3). Otospongiotic foci may involve not only the cochlea, but also the vestibule and the internal auditory canal. In our patient the entire cochlea and labyrinth were involved bilaterally. It is possible that labyrinthine involvement with otosclerosis contributed to our patient's vestibular findings. When the otosclerotic lesion is mature, it is rather difficult to recognize on CT, because its density makes it appear similar to the surrounding normal capsule. At this stage, an irregular outline of the capsule with scalloping of the endosteal and periosteal aspects of the otic capsule may be present on CT.[6]

The CT differential diagnosis of CO is short; only osteogenesis imperfecta is identical in appearance. In Paget's disease, in addition to cochlear lesions, lucent lesions appear in other parts of the calvarium and temporal bone. Dense mature otosclerosis should be differentiated from fibrous dysplasia. Generalized bony overgrowth does not occur in otosclerosis; when such overgrowth is present, the observer should consider fibrous dysplasia.[9]

In recent years, MRI with gadolinium enhancement has substituted for CT as the study of choice for retrocochlear workup of SNHL. Familiarity with MRI findings that may be consistent with CO is necessary so that the condition will not be overlooked in retrocochlear radiologic investigation. The MRI findings may be subtle and should be carefully sought. High-resolution CT is a more sensitive imaging study for the bony elements of the otic capsule. It should be used to confirm the diagnosis when CO is suspected.

There have been very few reports of MRI findings of otosclerosis: foci of otosclerosis have been reported as areas of pericochlear gadolinium enhancement.[7,10] This enhancement is attributed to the pooling of gadolinium in vascular spaces within active otosclerotic foci. It has been speculated that gadolinium enhancement may correspond to the activity of the otosclerotic focus.[10,11] On T1-weighted images, the lesions have been reported to be hyperintense.[10] T1-weighted images demonstrate the otosclerotic lesions better than T2-weighted images, because there is increased water content in spongiotic foci relative to normal capsular bone. Magnetic resonance imaging is inherently sensitive to flow and cellular water content. It has been shown that large foci are active and rich in vascular and nonmineralized fibrous tissue.[2] Therefore, MRI is more likely to display large and immature foci of otosclerosis than small and mature foci. In our case, MRI with gadolinium displayed a moderately intense signal in the pericochlear and perilabyrinthine bone that was most likely due to the extensive involvement of both otic capsules with otosclerotic spongiotic foci that had become confluent (Fig 2).

There have been a variety of theories explaining the cause of SNHL in otosclerosis. Spillage of enzymes from an active focus of otosclerosis into the cochlear fluid has been reported.[3] Those enzymes are toxic to the organ of Corti and, subsequently, to the spiral ligament. The enzymatic theory is the most widely accepted theory. Others have described venous shunts between the membranous labyrinth and the active otosclerotic bone.[12] Venous congestion secondary to those shunts was thought to cause cochlear hypoxia and hair cell malfunction. Balle and Linthicum[3] found hyalinization of the spiral ligament and atrophy of the stria vascularis adjacent to the otosclerotic foci. They speculated that there was an association between SNHL and those findings. They also described a correlation between the magnitude of SNHL and the severity of the atrophic changes in the spiral ligament. It is conceivable that more than one of these theories may have a role in explaining SNHL in CO. However, the magnitude of the SNHL and the size, degree of activity, and involvement of the endosteum by otosclerosis have shown no correlation in one study,[2] and a good correlation in another.[13] A correlation between the site of cochlear destruction on CT and the audiometric profile has been reported in some patients with mixed hearing loss and known stapedial otosclerosis. However, in those patients who have otosclerosis and a mixed hearing loss with lack of CT findings, it is presumed they have mature and mineralized foci that are not distinguishable on CT.[14]

One should be aware that reports of correlation between polytomographic findings suggestive of CO and temporal bone histology findings are conflicting: Britton and Linthicum[15] reported a single case in which a correlation did exist. They reported a patient with a mixed hearing loss and polytomographic evidence of cochlear otosclerotic involvement. The patient's temporal bone histology showed otosclerotic foci in the cochlea.[15] Applebaum and Shambaugh[16] reported low correlation of polytomographic findings and histologic findings in 3 pairs of temporal bones. In 1 of the 3 cases, the patient had pure SNHL and polytomographic findings suggestive of CO. However, temporal bone histology failed to reveal CO. They also found poor correlation between polytomographic and histologic findings in the other 2 pairs of temporal bones from patients with mixed hearing loss.[16] To the best of our knowledge, there has been no report of a positive correlation between polytomographic findings and histologic findings in pure CO. Further, there have been no reports in the literature to substantiate a correlation between CT findings and temporal bone histology in pure CO.

With our current understanding of CO and imaging technology, we are able to detect subtle or overt CT and MRI abnormalities in the cochlea in some patients with SNHL. It has also been shown that some patients with SNHL do have histologic evidence of CO. To date, however, there is no scientific evidence to link the radiologic findings with the histologic findings of isolated CO.

A great deal of caution should therefore be exercised by clinicians and radiologists when interpreting radiologic findings as consistent with CO. There is an equally possible chance that the same radiologic findings may represent one of a variety of other disorders, all of which cause demineralization of the otic capsule. This admonition must be applied to the case presented here as well. This is not to say that high-resolution CT should not be obtained for patients with SNHL who are at risk for CO. Rather, we suggest obtaining CT scans of these patients in order to increase our data pool. With more data, the appropriate scientific correlations can be determined.

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CLINICOPATHOLOGICAL CONSULTATION

SYNOVIAL SARCOMA OF THE LARYNX AND HYPOPHARYNX

ANGELO P. DEI TOS, MD
TREVISO, ITALY

PAOLA DAL CIN, PHD
LEUVEN, BELGIUM

RAF SCIOT, MD
LEUVEN, BELGIUM

ALBERTO FURLANETTO, MD
TREVISO, ITALY

M. CRISTINA DA MOSTO, MD
TREVISO, ITALY

CATERINA GIANNINI, MD
TREVISO, ITALY

ALESSANDRA RINALDO, MD
PADUA, ITALY

ALFIO FERLITO, MD
UDINE, ITALY

Synovial sarcoma represents a mesenchymal malignancy of unknown histogenesis that most often occurs in the lower limbs of young adults. The head and neck region is a relatively rare location, in which the hypopharynx and larynx are, respectively, the most and least often affected anatomic sites. Histologically, synovial sarcomas are classified into monophasic and biphasic variants. Immunohistochemistry plays a major part in the differential diagnosis, enabling the demonstration of epithelial differentiation. Both monophasic and biphasic synovial sarcomas are characterized cytogenetically by the reciprocal translocation t(X;18)(p11.2;q11.2) between chromosomes X and 18. Two cases of synovial sarcoma arising in the larynx and in the hypopharynx and in which cytogenetic analysis detected a diagnostic t(X;18) chromosome aberration are reported here.

INTRODUCTION

Synovial sarcoma is a mesenchymal malignancy and accounts for approximately 10% of all soft tissue sarcomas.[1] It usually occurs in the lower limbs of young adults, and only 3% of all synovial sarcomas involve the head and neck region. While the hypopharynx is involved in most cases arising in the head and neck area, primary laryngeal synovial sarcoma seems to be exceptional.[2] Its histogenesis has remained a matter of debate. Considering its tendency to occur close to major articular structures, a relationship with synovium has been postulated. However, the concept that synovial sarcoma exhibits no evidence of true synovial differentiation has now been widely accepted.[3]

The clinicopathological findings in 2 cases of synovial sarcoma, 1 occurring in the larynx and 1 in the hypopharynx, are presented, together with the outcome of cytogenetic analysis.

CASE REPORTS

Case 1. A 27-year-old man presented to the Department of Otolaryngology of the Catholic University of Leuven, Belgium, with a history of relapsing pharyngitis. Direct laryngoscopy revealed a mass in the right aryepiglottic fold, and after biopsy, surgical resection of the tumor was performed. The lesion was diagnosed as synovial sarcoma. Local recurrence of the neoplastic lesion presented after 3 months of follow-up. Chemotherapy (high doses of ifosfamide) and subsequent radiotherapy were applied, but a right hemilaryngectomy was performed 3 months later due to persisting tumor growth. There was a 2-cm tumor in the pyriform sinus, with no invasion of the laryngeal skeleton. The patient is alive at 9 months of follow-up.

Case 2. A 35-year-old male drug abuser first presented to the Department of Surgery in Treviso Hospital with dysphagia. Flexible endoscopy revealed a mass in the cervical esophagus, the presence of which was not confirmed during surgical exploration, though the symptoms disappeared following surgery. The patient presented to the Intensive Care Unit, however, because of acute respiratory failure due to heroin overdose. During intubation, an exophytic mass located in the right lateral wall of the hypopharynx was observed. Direct pharyngolaryngoscopy was then performed, and the lesion was submitted to biopsy. The diagnosis was synovial sarcoma. The patient

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From the Department of Pathology (Dei Tos, Furlanetto, Giannini) and the Division of Otolaryngology (Da Mosto), Regional Hospital, Treviso, the Department of Otolaryngology, University of Padua, Padua (Rinaldo), and the Department of Otolaryngology–Head and Neck Surgery, University of Udine, Udine (Ferlito), Italy, and the Center for Human Genetics (Dal Cin) and the Department of Pathology (Sciot), Catholic University, Leuven, Belgium. This paper is supported by the Lega Italiana Contro i Tumori (Treviso Section), the Belgian Inter-University Poles of Attraction Program (initiated by the Belgian State, Prime Minister's Office, Science Policy Programming), and The Laryngeal Cancer Association, Italy.

CORRESPONDENCE — Alfio Ferlito, MD, Dept of Otolaryngology–Head and Neck Surgery, University of Udine, Policlinico Città di Udine, Viale Venezia 410, 33100 Udine, Italy.

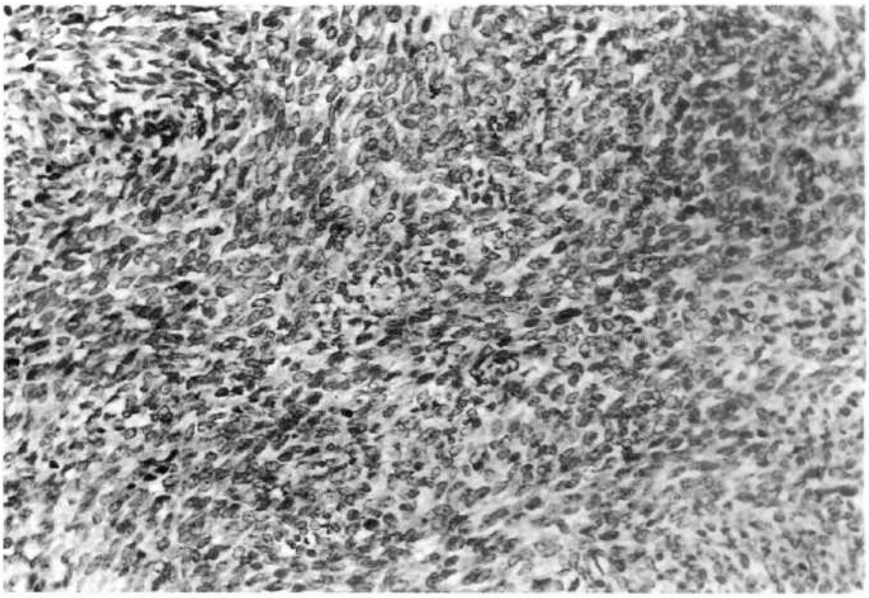

Fig 1. (Case 1) Synovial sarcoma (H & E, original ×200). Monomorphic spindle cell proliferation showing tendency to grow in fascicular pattern.

underwent chemotherapy (ifosfamide, epirubicin, dacarbazine) followed by radiotherapy (6,200 cGy). Complete remission of the primary lesion was observed, but after 5 months of follow-up, computed tomography of the chest revealed a mass in the upper lobe of the right lung that was interpreted without biopsy as a distant metastasis. The patient is alive with disease at 10 months of follow-up.

MATERIALS AND METHODS

In both cases, 4-μm sections were cut from formalin-fixed and paraffin-embedded surgical specimens and stained with hematoxylin and eosin.

The mitotic rate was expressed as the highest mitotic count found in 10 high-power fields (HPFs; 1 HPF = 0.159 mm^2 on the microscope used).

Immunohistochemistry. Sections were immunostained for cytokeratin (clones CAM5.2, Becton-Dickinson, Mountain View, Calif, dilution 1:20, and AE1/A3, BioGenex, Carpinteria, Calif, dilution 1:200); vimentin (clone V9, Dako, Glostrup, Denmark, dilution 1:400); epithelial membrane antigen (EMA; clone E29, Dako, dilution 1:100); desmin (clone D33, Dako, dilution 1:200); smooth muscle actin (clone 1A4, Sigma, dilution 1:4,000); S-100 protein (polyclonal, Dako, dilution 1:2,000); and CD99 (clone 013, Signet, Dedham, Mass, dilution 1:50). Immunostains were performed by a peroxidase technique on a GEN II automated immunostainer (Ventana, Tucson, Ariz).

Cytogenetics. Fresh tissue was obtained from the tumor under sterile conditions for cytogenetic analysis. Twenty metaphases were obtained after short-term culture from an overnight collagenase-disaggregated sample of the tumor.

RESULTS

Microscopically, case 1 was characterized by the presence of a highly cellular spindle cell proliferation, set in collagenous stroma, that showed a tendency to grow beneath an intact squamous epithelium. The spindle cells formed intersecting fascicles with no tendency toward the formation of epithelial-like structures (Fig 1). Nuclei were rather monomorphic. Mitotic figures were scarce, and ranged between 1 and 3 mitoses per 10 HPF. In case 2, in addition to a spindle cell proliferation showing the same histologic findings as in case 1, there were clusters of epithelioid cells that gave the tumor a strikingly biphasic pattern (Fig 2A). Rare mast cells were present in both cases.

Expression of cytokeratin (Fig 2B), EMA, vimentin, and CD99 was observed in both cases. In case 1, cytokeratin and EMA immunostaining was limited to focal groups of spindle cells. In case 2, both antibodies decorated the epithelioid component, whereas spindle cells exhibited focal immunoreactivity. CD99 decorated both spindle and epithelioid cells. Desmin and smooth muscle actin stains were negative, while focal S-100 immunopositivity was observed in the spindle cell component in case 2.

Cytogenetic analysis revealed a 46,Y,t(X;18)(p11.2; q11.2) in both cases (Fig 2C). A diagnosis of monophasic and biphasic synovial sarcoma was made in cases 1 and 2, respectively.

DISCUSSION

Synovial sarcoma represents a mesenchymal malignancy that is clinically characterized by occur-

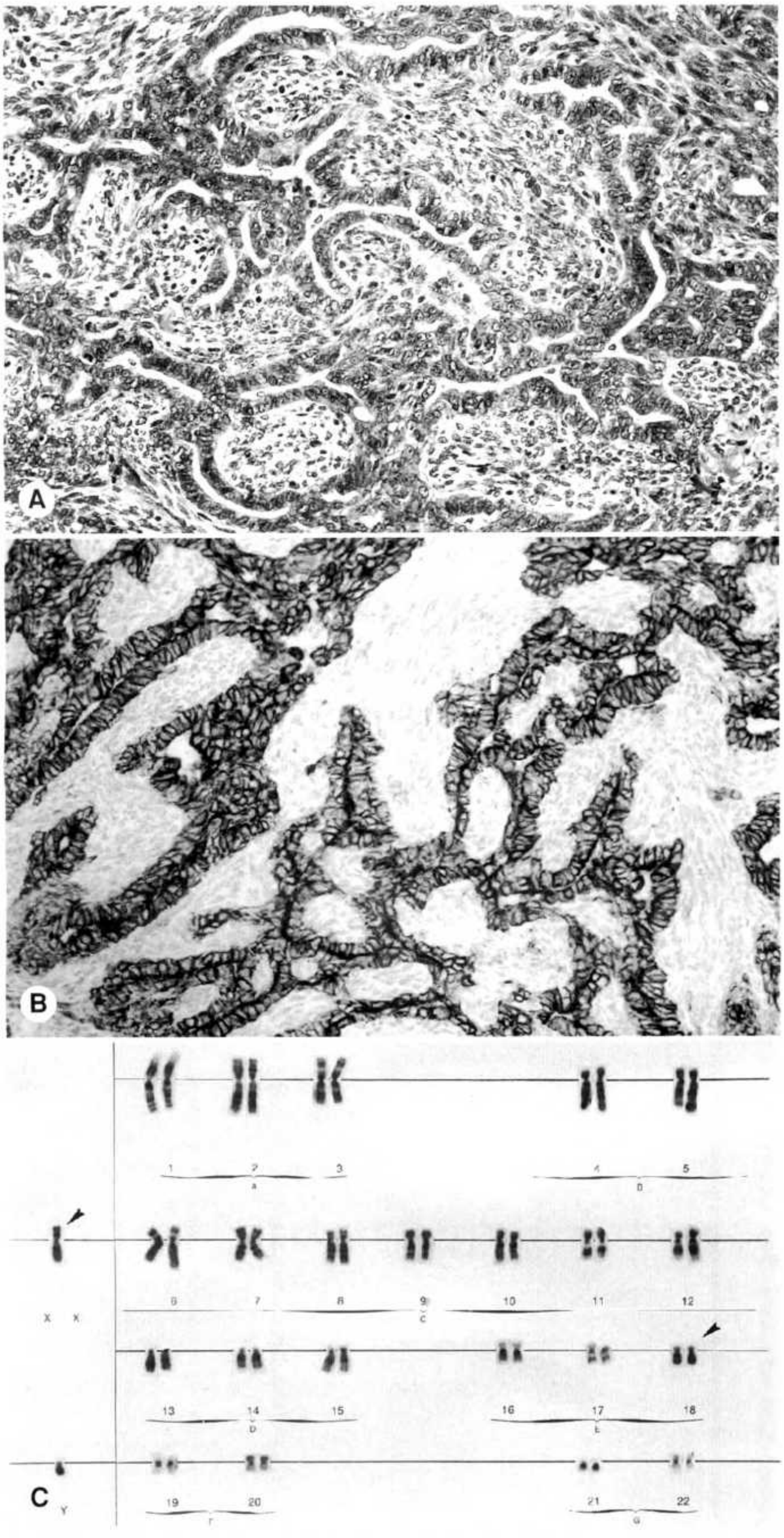

Fig 2. (Case 2) Synovial sarcoma. A) Epithelial-like structures intermingled with spindle cell proliferation represent histologic hallmark of biphasic synovial sarcoma (H & E, original ×200). B) Presence of glandular structures is highlighted by cytokeratin immunostaining (immunoperoxidase, original ×400). C) G-banded metaphase showing reciprocal [t(X;18)(p11.2;q11.2); arrowheads] — characteristic chromosome change in synovial sarcoma.

rence in the lower limbs of young adults, with a peak incidence ranging between the second and third decades. A more rare, but well-characterized site of occurrence is the head and neck region, with about 100 reported cases, the hypopharynx being the most frequently affected site. By contrast, the larynx appears to be an exceptional location, with just 10 convincing cases.[4-7]

Two main variants of synovial sarcoma are recognized: the monophasic and the biphasic subtypes. Both variants feature a spindle cell population set in a variable collagenous background and associated with a hemangiopericytoma (HPC)–like vascular pattern (ie, a vascular network composed of branching and dilated thin-walled blood vessels). In the biphasic subtype, glandularlike structures are also found. In many circumstances, gland formation can be very subtle and represented merely by the clustering of cuboidal cells that are more easily identified by means of both reticulin stains and immunohistochemistry. Exceptionally, a predominantly monophasic epithelial pattern has been reported.[8] Rare undifferentiated cases exhibit a monomorphic round cell morphology that represents a true diagnostic challenge.

Synovial sarcoma is characterized by a complex and relatively distinctive immunophenotype that includes coexpression of mesenchymal (vimentin) and epithelial markers (cytokeratins, EMA). As morphologic features of epithelial differentiation may be very subtle, these findings represent a truly valuable diagnostic aid in the differential diagnosis with respect to other spindle cell sarcomas such as fibrosarcoma and malignant peripheral nerve sheath tumor (MPNST). It has to be stressed that EMA as well as cytokeratin expression in spindle cell monophasic synovial sarcoma may be focal. Careful examination of different sections is therefore recommended to avoid diagnostic errors. CD99 and S-100 protein immunoreactivity, which represent potential diagnostic pitfalls, have also recently been reported in both spindle and epithelioid cell components of synovial sarcoma.[9,10] CD99 is known to be a fairly sensitive marker for the Ewing's sarcoma–peripheral neuroectodermal tumor category, a spectrum of mesenchymal malignancies exhibiting a partial neuroectodermal phenotype that belong to the so-called family of pediatric small round cell tumors (including alveolar rhabdomyosarcoma, neuroblastoma, and lymphoblastic lymphoma). Since synovial sarcoma, when it occurs in its poorly differentiated form, tends to assume a rather undifferentiated, round cell morphology, awareness of the commonly observed CD99 immunopositivity, along with its evaluation in context with epithelial differentiation markers, will avoid diagnostic confusion. Some synovial sarcomas may stain with carcinoembryonic antigen and human milk fetal globule protein in the epithelial component.[7]

The expression of S-100 protein, observed in about 30% of cases of synovial sarcoma, does not per se rule out the diagnosis of MPNST. Malignant PNST may arise sporadically or in the context of the neurofibromatosis 1 syndrome, and is characterized by a fascicular spindle cell proliferation that tends to exhibit a peculiar perivascular accentuation of cellularity. Immunohistochemically, no more than 50% of MPNSTs show S-100 protein immunopositivity in less than 30% of pathologic cells, thus showing a considerable immunophenotypic overlap with synovial sarcoma. With the exception of the epithelioid variant, MPNST is cytokeratin-negative. Nonetheless, it has to be stressed that EMA immunopositivity can be detected as a result of perineural differentiation.[11]

When dealing with monophasic synovial sarcoma, an important differential diagnosis concerns solitary fibrous tumor, which has been increasingly reported at extrapleural sites, including the upper respiratory tract.[12] Solitary fibrous tumor shares with synovial sarcoma the presence of an HPC-like vascular pattern, as well as the overexpression of bcl-2, a molecule that plays a major part in the negative control of apoptotic processes.[13] The presence of a patternless spindle cell proliferation exhibiting a varying cellularity and a strong CD34 immunopositivity along with keratin-EMA negativity permits differential diagnosis in most instances.

The fact that any presence of an HPC-like vascular network is one of the commonly used diagnostic criteria necessarily includes in the differential diagnosis the enigmatic "entity" christened by Stout and Murray[14] in 1942 as HPC. Twelve cases of HPC have reportedly occurred in the larynx.[2] In addition to the fact that even in Stout's original description this lesion was more a diagnosis of exclusion, it is worth emphasizing that the theory that HPC may represent a histologic pattern rather than a true clinicopathological entity is gaining acceptance.[15] It may be that in the future, HPC as conceived by Stout and Murray will be incorporated by other entities such as infantile myofibromatosis, solitary fibrous tumor, and synovial sarcoma.[3,12,15,16]

Synovial sarcoma needs to be distinguished from spindle cell carcinoma (so-called carcinosarcoma). Spindle cell carcinoma generally presents grossly as an ulcerated, polypoid mass that is histologically composed mainly of a spindle cell proliferation that may closely mimic a sarcoma. The observed coexpression of cytokeratins and EMA is not sufficient in differentiating spindle cell carcinoma from synovial sarcoma. Nonetheless, careful sampling of the surgical specimen usually permits the detection of areas featuring conventional squamous cell carcinoma. Yet, it has to be emphasized that foci of squamous differentiation may be occasionally found in otherwise typical biphasic synovial sarcomas.[1]

Cytogenetically, both monophasic and biphasic synovial sarcomas are characterized by the reciprocal translocation t(X;18)(p11.2;q11.2),[17] which leads at a molecular level to fusion between the gene called SYT (for synovial sarcoma translocation) on chromosome 18 and the genes called SSX1 (for synovial sarcoma X breakpoint) and SSX2 on the X chromosome.[18-20] The SYT-SSX1 translocation is associated with the biphasic type.[21] The presence of this specific chromosome aberration represents a useful diagnostic tool, particularly in dealing with poorly differentiated tumors or lesions arising at unusual sites.[22] The SYT gene is unrelated to any other known gene, but contains a predicted glutamine-proline-glycine–rich region suggestive of a transcriptional activation domain. The SSX1 and SSX2 genes are also unrelated to other known genes and encode proteins that show a remarkable homology. The translocation leads to the formation of a chimerical transcript, as in other sarcoma translocations.[23] This fusion gene model represents an optimal target for both cytogenetics and molecular pathology, which can detect the translocation by applying chromosome analysis or the reverse transcriptase–polymerase chain reaction technique. As far as the molecular cytogenetics of soft tissue sarcomas is concerned, it must be emphasized that the fluorescence in situ hybridization technique seems particularly promising.[24] Such a novel diagnostic approach to synovial sarcoma may prove truly helpful in certain conditions. In fact, while the diagnostic approach to soft tissue sarcomas arising in surgically amenable soft tissues is greatly facilitated by the availability of abundant pathologic tissue, when dealing with tumors located in the laryngeal-hypopharyngeal area, a morphologic diagnosis must often be rendered on the basis of histologic findings in small bioptic fragments. In this context, a purely morphologic diagnosis may represent a challenge. Both classical and molecular cytogenetic analyses therefore represent particularly useful ancillary diagnostic techniques that, because of their specificity, enable diagnostic confusion to be avoided. A combination of fine-needle aspiration and specific cytogenetic abnormality ensures definitive diagnosis.[25]

Molecular analysis not only represents an important aid to morphologic diagnosis, but can also provide important independent prognostic information. Not only does the SYT-SSX2 fusion transcript correlate with the monophasic phenotype, it also is associated with a significantly better metastasis-free survival.[21] In fact, the differential diagnosis of synovial sarcoma, which includes other mesenchymal malignancies as well as spindle cell carcinoma, may often be challenging, and the availability of highly sensitive and specific ancillary diagnostic techniques, such as classical and molecular cytogenetics, represents a major advance in the management of these rare malignancies.

The recognition of synovial sarcoma is also of paramount importance in ensuring the right therapeutic approach. The discovery of synovial sarcoma's chemosensitivity to ifosfamide compounds has represented an important clinical advance in the therapeutic approach to adult soft tissue sarcomas.[26] This is even more relevant when dealing with lesions arising at locations such as the larynx and hypopharynx, for which radical surgical treatment may be either difficult to achieve or lead to severe functional loss. Lymph node metastasis has not been reported from laryngeal synovial sarcoma, and is quite exceptional in hypopharyngeal lesions, so prophylactic neck dissection is not indicated. Recurrent or metastatic synovial sarcoma may show histologic variation from the primary neoplasm; ie, a biphasic tumor may metastasize or recur as a monophasic lesion. Given the rarity of these lesions, data concerning the value of a multitherapeutic approach are still limited.[27] Nonetheless, the chances of establishing a more rational management of synovial sarcoma depend upon its recognition and correct classification.

In order to ensure a proper therapeutic approach, the distinction of synovial sarcoma from other mesenchymal malignancies or spindle cell carcinoma is mandatory, and the application of ancillary diagnostic techniques is therefore recommended.

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BOOK REVIEWS

Rhinology and Sinus Disease. A Problem Oriented Approach

Steven D. Schaefer, editor. Hard cover, illustrated, indexed, 285 pages, 1998. Mosby, St Louis, Mo, $75.

Purpose: The stated purpose of this text is to present a case-oriented approach to rhinology and the management of sinus disease in the context of the many changes that have occurred in this area over the last 2 decades. It seeks to present a variety of approaches and opinions from numerous well-respected authors.

Contents: This text covers the gamut of topics related to the nose and sinuses, from imaging and endoscopy to allergy, sinusitis, neoplasm, and trauma. Rhinologic subjects, including nasal obstruction and septoplasty, epistaxis, laser surgery, and management of the obstructing inferior turbinate, are highlighted, with the addition of some more esoteric subjects, including choanal atresia, thyroid ophthalmopathy, and dacryocystorhinostomy. Chapters devoted to the management of sinusitis and asthma are divided up, not only according to the sinuses involved, but also into pediatric and adult chapters. Frank discussions of the relative attributes of both traditional and endoscopic approaches provide well-integrated, practical strategies for common and infrequent sinus problems. Three separate chapters are devoted to sphenoethmoidectomy and ethmoidectomy, adding diversity and allowing the reader to explore the similarities of these different approaches.

Attributes: Most of the surgical discussions in the book provide the type of detailed description of technique one would expect from a surgical atlas. Many of the authors have shared both published and unpublished data from their own vast experiences, as well as some of the "pearls" of management that are frequently only shared in a "breakfast with the faculty" type of setting. Controversial areas, such as resection of the middle turbinate, are handled in an objective fashion. The case-oriented approach was adhered to through most of the chapters, providing a specific application of the otherwise didactic material.

Limitations: Unpublished data and personal experience are of inestimable value in medicine, but escape the editorial review required for academic journal publication. The problem-oriented approach of this text lends itself to discussions of personal preference and experience, but also introduces a less rigid standard for the data presented.

Applicability: This text provides up-to-date discussions of the spectrum of rhinologic and sinus disease, is well illustrated, and would be a useful addition to the library of most otolaryngologists who care for patients with sinus disease.

Donald J. Wittich, Jr, MD
Birmingham, Alabama

Hyperbaric Oxygen Therapy in Otorhinolaryngology

Noriyuki Yanagita and Tsutomu Nakashima, editors. Volume 54 of *Advances in Oto-Rhino-Laryngology*. Hard cover, illustrated, indexed, 129 pages, 1998. Karger, Basel, Switzerland.

Purpose: Up-to-date important information for the clinical use of hyperbaric oxygen therapy (HBO) in otolaryngology–head and neck surgery (ENT).

Contents: Can be divided into 1) indications of HBO in general medicine, 2) treatment of ENT conditions (necrotizing cervical infections, noise-damaged inner ears, sudden hearing loss, facial palsy), and 3) otological complications of HBO.

Attributes: The effectiveness (or lack of it) of HBO therapy in treating ENT disorders is described in detail with well-received and conclusive information. In summary, conveyed to the reader is information supporting the statements that HBO is recommended and warranted, time-wise, in those patients with idiopathic sudden deafness, acoustic trauma, or noise-induced hearing loss within 3 months after onset of the disorder; that in animal studies, HBO combined with prednisone gives the best therapeutic effect in hypoxic and ischemic inner ears; and that HBO should be considered in all patients with cervical necrotizing fasciitis.

Limitations: Lack of specificity for indications of treatment with HBO for certain conditions, eg, sudden deafness. Various factors are associated with the outcome of sudden deafness, such as degree of hearing loss, low frequency or high frequency, severity of hearing loss, etc. When we use a specific treatment such as HBO for all aspects of hearing loss from sudden deafness, then HBO may be merely another empirical treatment.

Applicability: The book contributes to our understanding of this treatment strategy. Otolaryngologist–head and neck surgeons will find this summary of the current state of knowledge of HBO useful and informative.

Dennis G. Pappas, MD
Birmingham, Alabama

Essential Audiology for Physicians

Kathleen Campbell. Soft cover, illustrated, indexed, 191 pages, 1998. Singular Publishing Group, San Diego, Calif, $29.95.

Purpose: To familiarize physicians, resident and practicing, with the role of the audiologist in the diagnosis and rehabilitation of individuals with disorders affecting the auditory system.

Contents: Routine audiometric testing of children and adults; special audiometric tests for site-of-lesion determination, hearing aid evaluation, or rehabilitation of hearing aid users and cochlear implant recipients.

Attributes: Clearly describes the routine types of audiometric tests used in assessing common types of hearing loss and also discusses the more specialized types of tests used in site-of-lesion diagnosis. Sections on hearing aid evaluation and cochlear implantation discuss the limitations and the positive aspects of what might be anticipated from this type of rehabilitation.

Bibliography: There is no formal bibliography; however, at the end of each chapter there is a list of references for further reading.

Applicability: The information contained in this book is of interest not only to audiologists but also to any physician dealing with patients who might have a hearing problem, including primary care physicians, pediatricians, internists, neurologists, and neurosurgeons.

Fred H. Linthicum, Jr, MD
Los Angeles, California

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